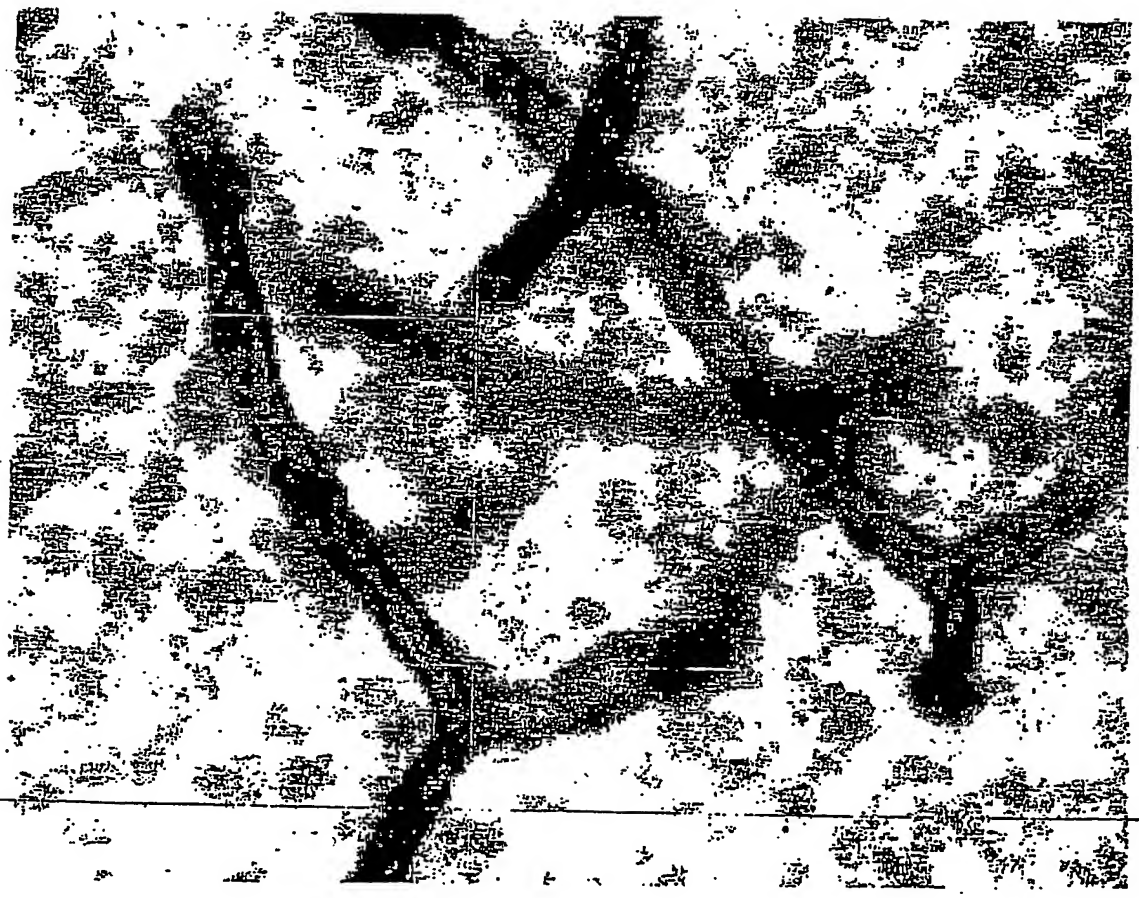
Figure 1

Capillaries of the human brain express very high levels of EPO receptor, as determined by immunohistochemistry using specific anti-EPO receptor antibodies. This provides a mechanism whereby EPO is able to penetrate into the brain from the systemic circulation, in spite of the blood brain barrier.

Figure 2



EPO receptor is densely localized within and around capillaries forming the blood brain barrier in the human brain

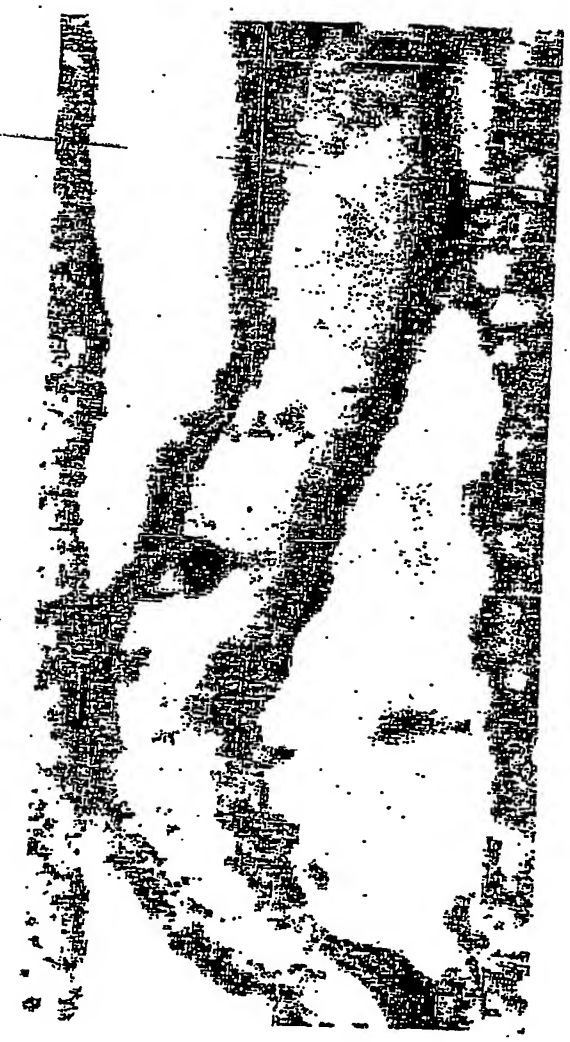
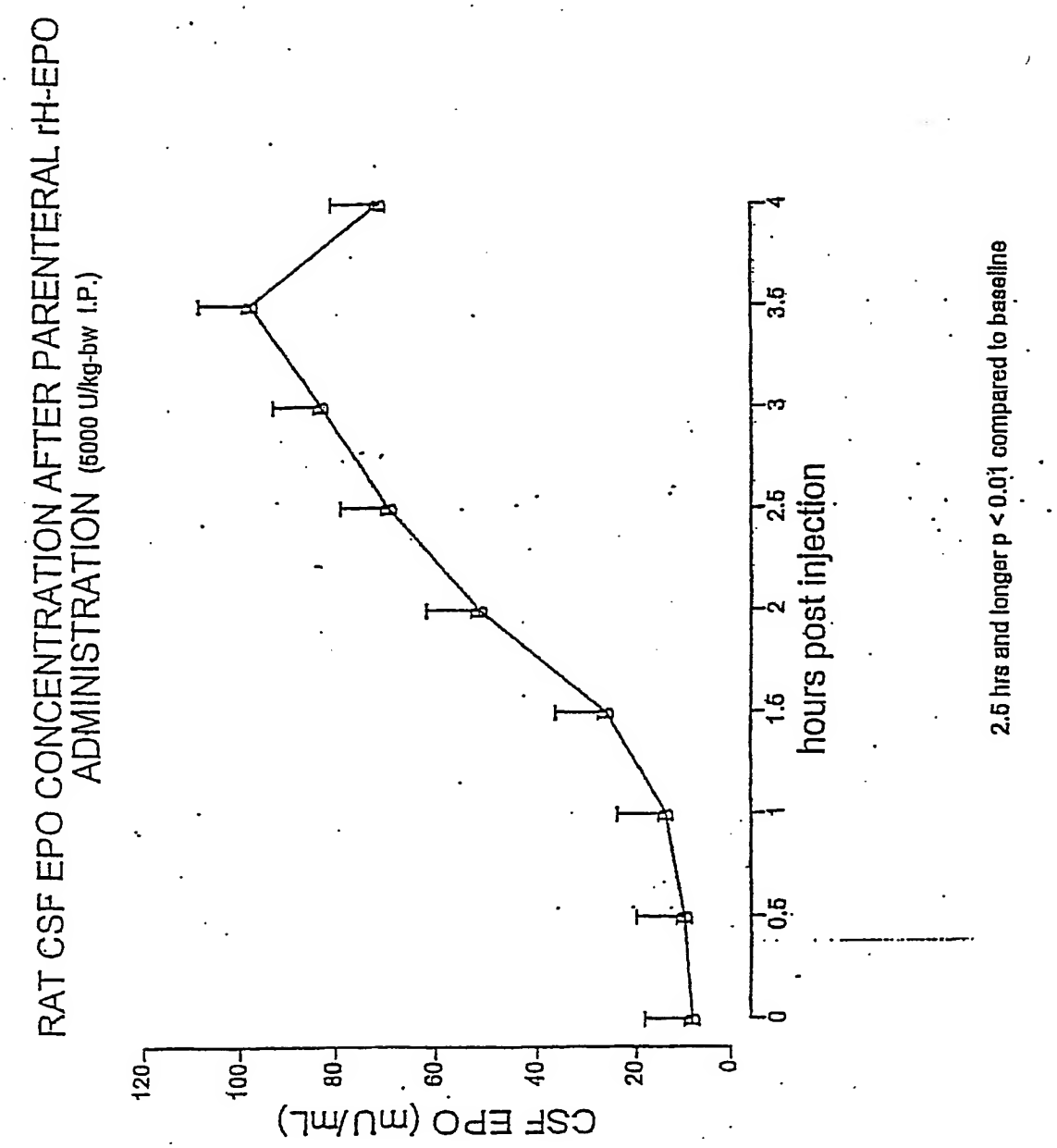

High density of EPO receptor is at the luminal and anti-luminal surfaces of human brain capillaries, forming the anatomical basis for transport of EPO from the circulation into the brain.

Figure 3

EPO receptor is found upon the endothelial surface (*), within cytoplasmic vesicles (arrows) and upon glial endfeet (+) in human brain, providing the anatomical basis for transport of EPO from within the circulation into the brain.

Figure 4

Figure 5


RAT CSF EPO CONCENTRATION AFTER PARENTERAL rH-EPO ADMINISTRATION (6000 U/kg-bw I.P.)
CSF EPO (mU/mL)
0
20
40
60
80
100
120
0
0.5
1
1.5
2
2.5
3
3.5
4
hours post injection
2.5 hrs and longer p < 0.01 compared to baseline

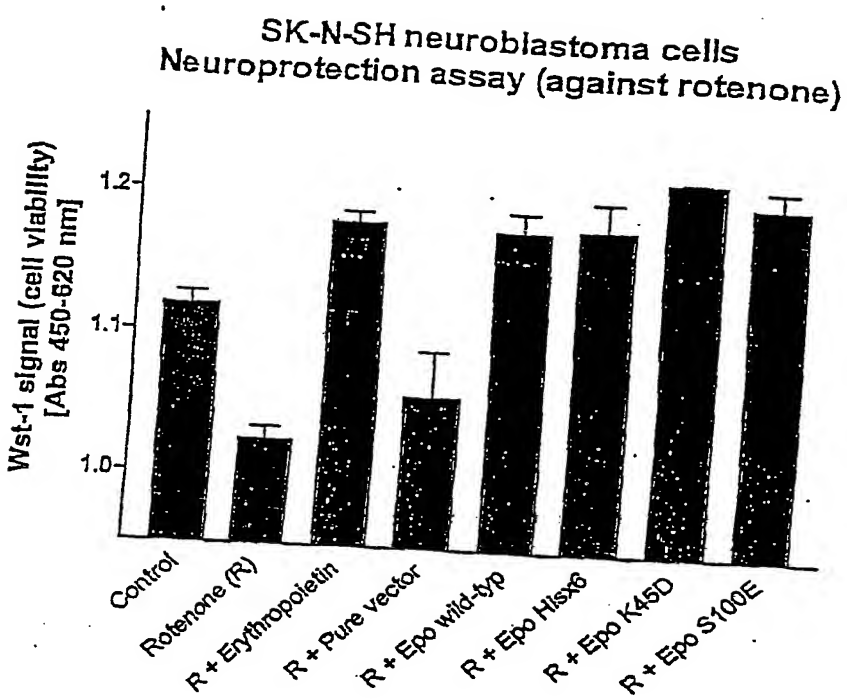

SK-N-SH neuroblastoma cells
Neuroprotection assay (against rotenone)
Wst-1 signal (cell viability)
[Abs 450-620 nm]
1.2
1.1
1.0
Control
Rotenone (R)
R + Erythropoietin
R + Pure Vector
R + Epo wild-typ
R + Epo Hisx6
R + Epo K45D
R + Epo S100E

Figure 6A

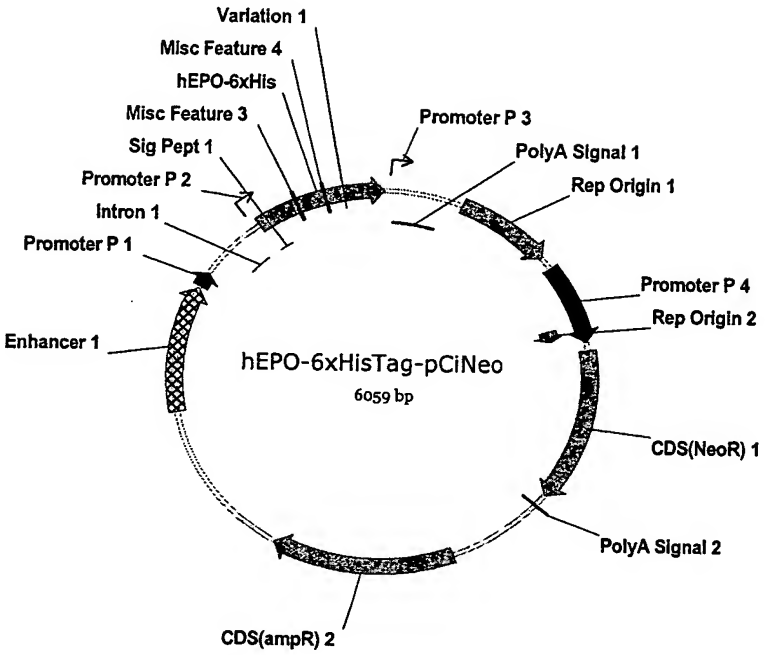

Variation 1
Misc Feature 4
hEPO-6xHis
Misc Feature 3
Sig Pept 1
Promoter P 2
Intron 1
Promoter P 1
Enhancer 1
Promoter P 3
PolyA Signal 1
Rep Origin 1
Promoter P 4
Rep Origin 2
CDS(NeoR) 1
PolyA Signal 2
CDS(ampR) 2
hEPO-6xHisTag-pCiNeo
6059 bp

Figure 6B

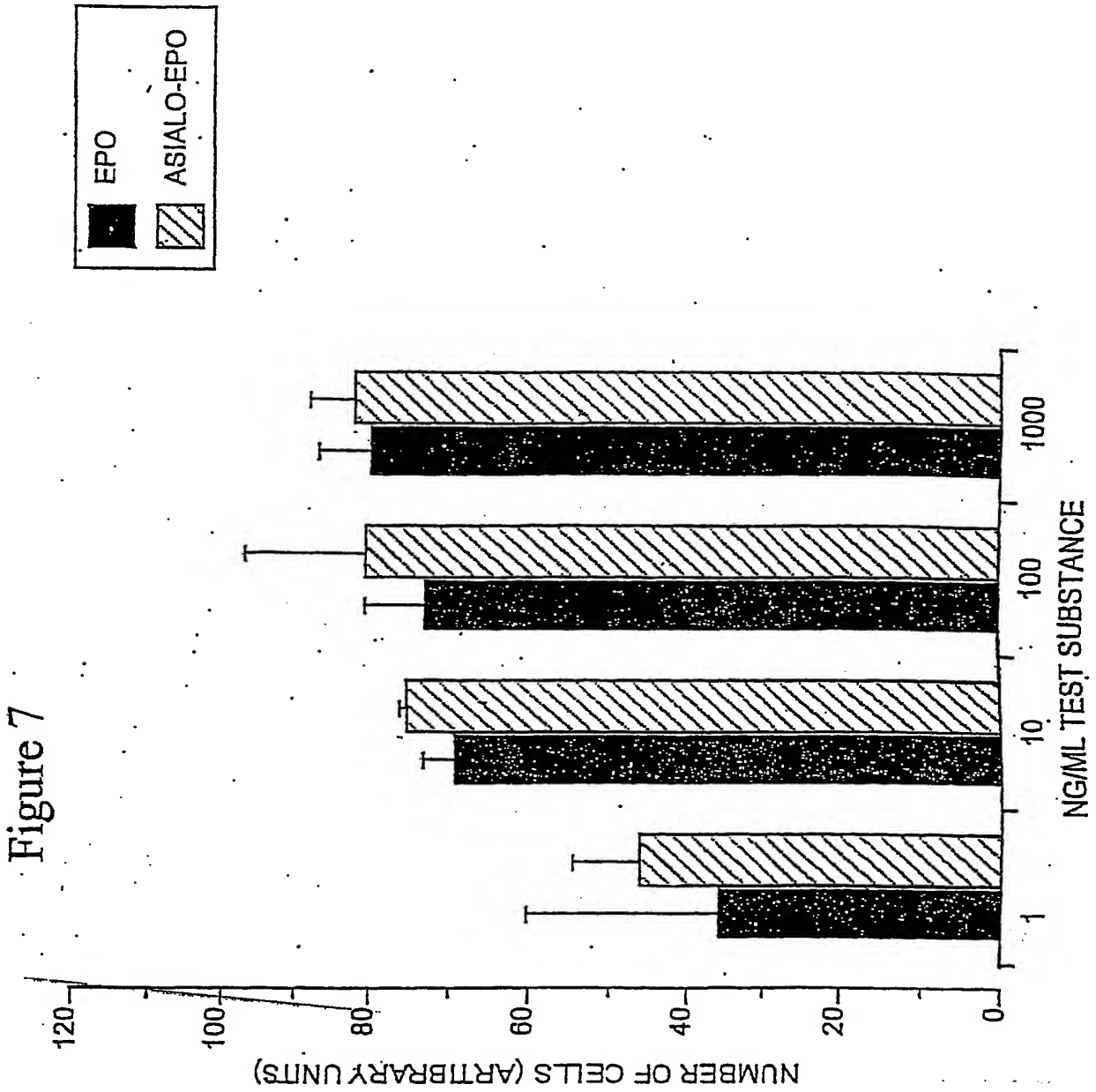

EPO
ASIALO-EPO
NUMBER OF CELLS (ARTIBRARY UNITS)
0
20
40
60
80
100
120
1
10
100
1000
NG/ML TEST SUBSTANCE

Figure 7

Figure 8

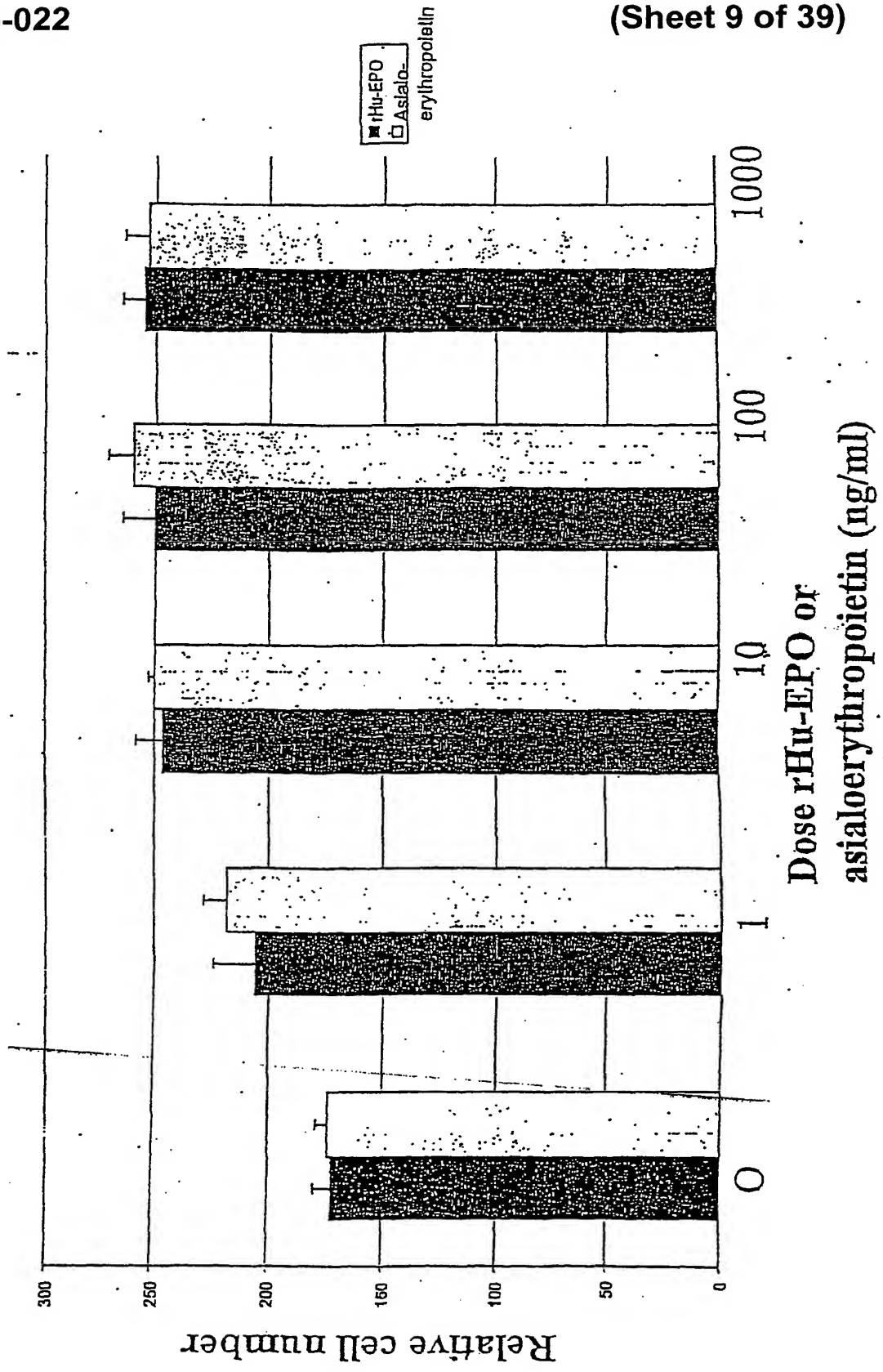

rHu-EPO
Asialo-erythropoietin
Relative cell number
300
250
200
150
100
50
0
0
1
10
100
1000
Dose rHu-EPO or asialoerythropoietin (ng/ml)

Figure 9

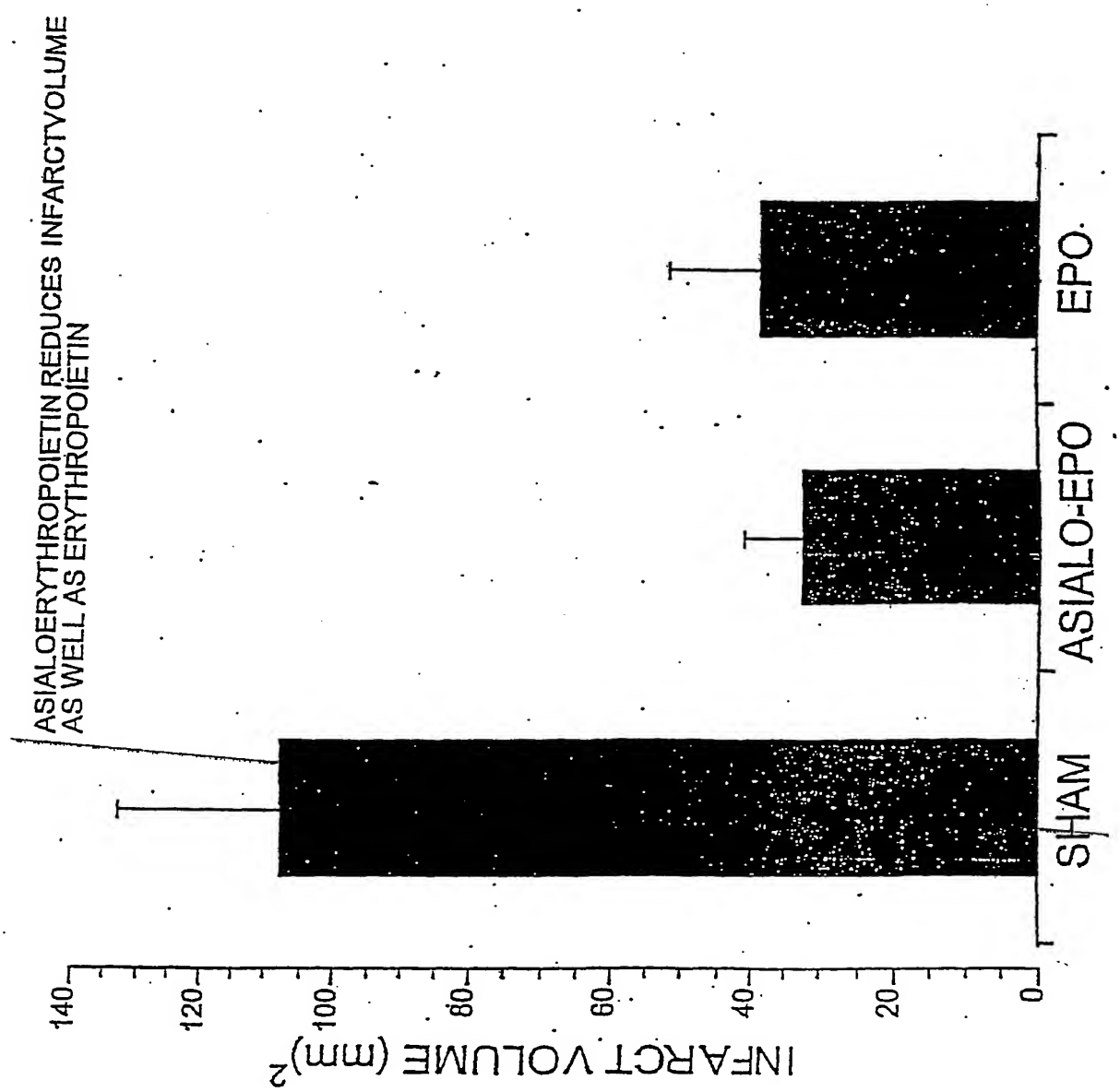

ASIALOERYTHROPOIETIN REDUCES INFARCTVOLUME
AS WELL AS ERYTHROPOIETIN
INFARCT VOLUME (mm)2
140
120
100
80
60
40
20
0
SHAM
ASIALO-EPO
EPO

Figure 10

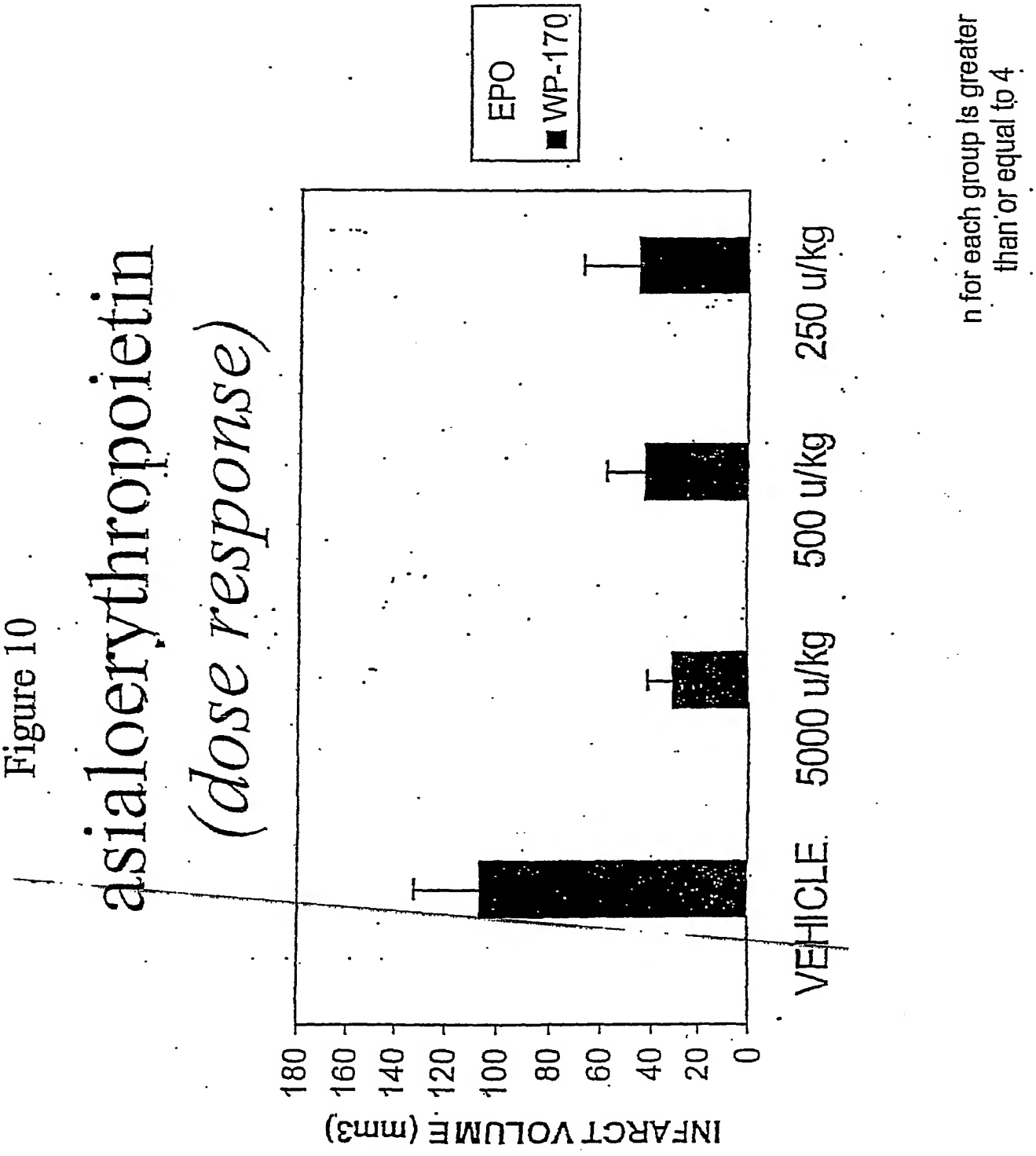

asialoerythropoietin
(dose response)
EPO
WP-170
INFARCT VOLUME (mm3)
0
20
40
60
80
100
120
140
160
180
VEHICLE
5000 u/kg
500 u/kg
250 u/kg
n for each group is greater than or equal to 4

Figure 11

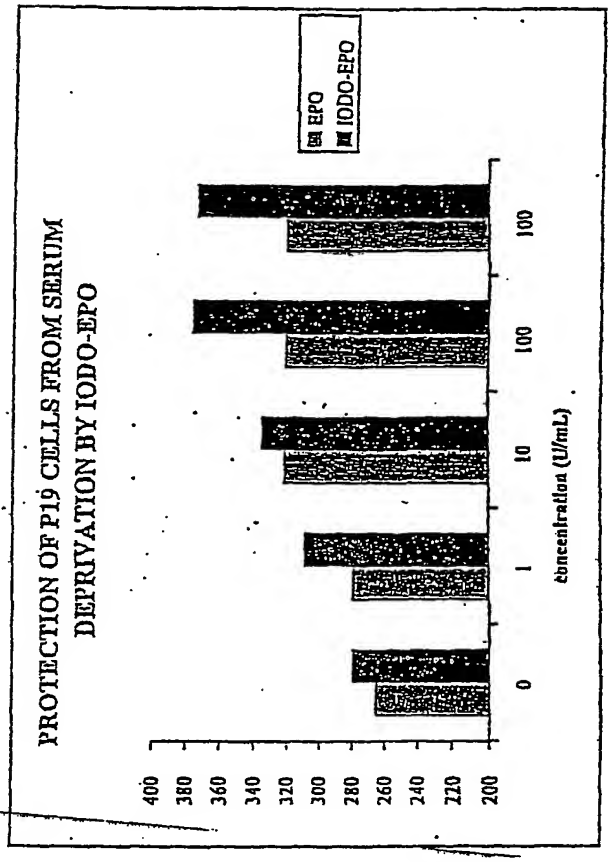

PROTECTION OF P19 CELLS FROM SERUM DEPRIVATION BY IODO-EPO
EPO
IODO-EPO
400
380
360
340
320
300
280
260
240
220
200
0
1
10
100
100
Concentration (U/mL)

Figure 12

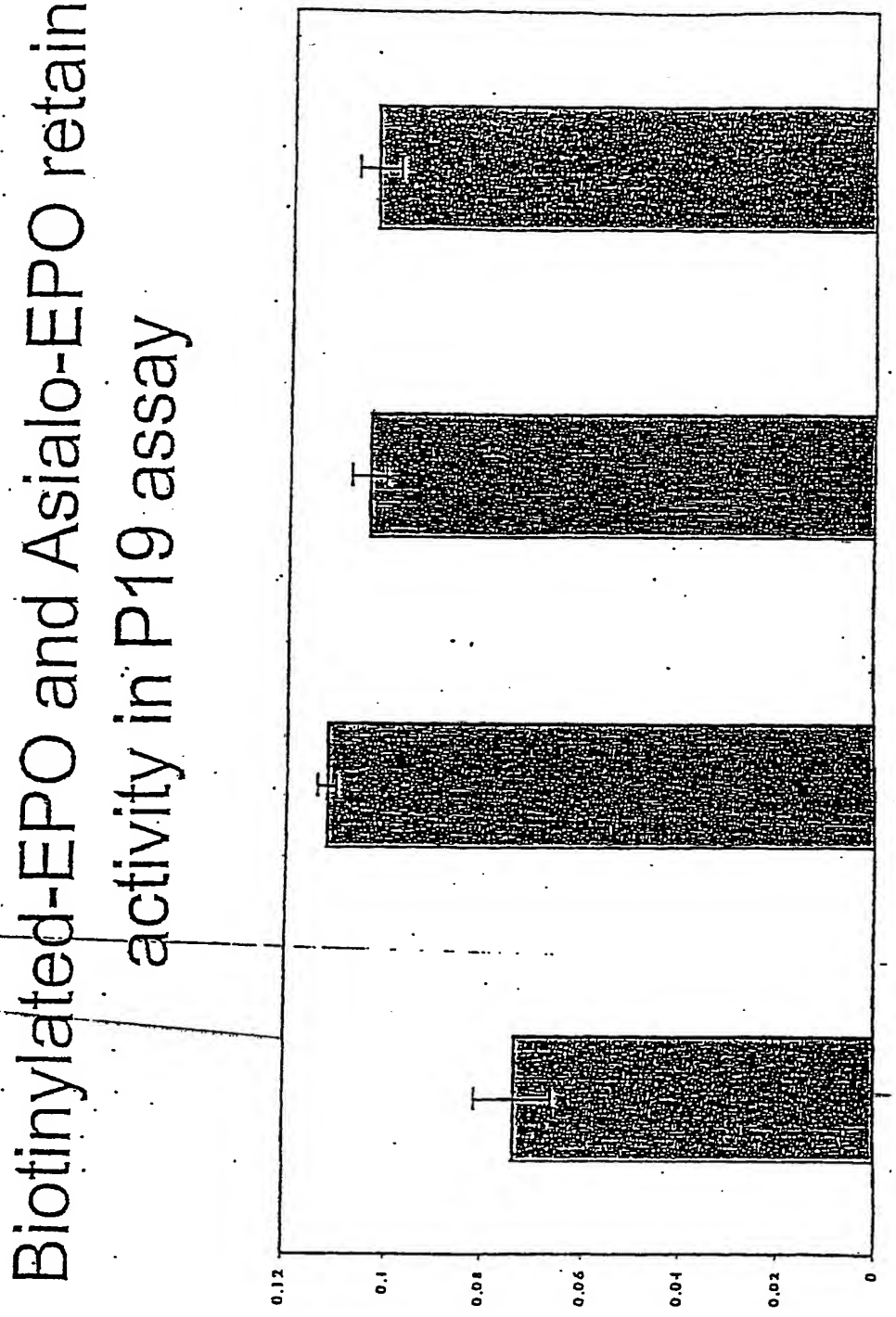

Biotinylated-EPO and Asialo-EPO retain activity in P19 assay
0.12
0.1
0.08
0.06
0.04
0.02
0

Figure 13

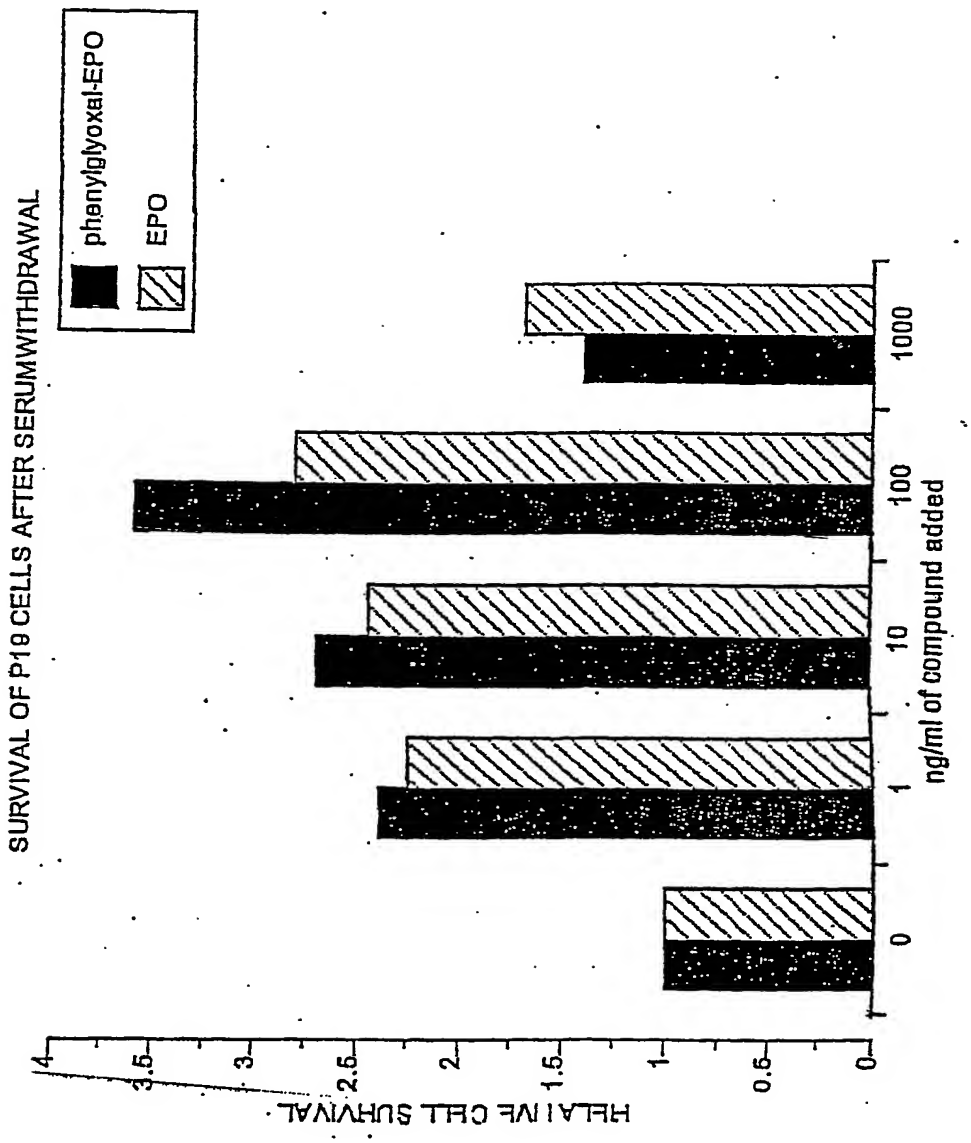

SURVIVAL OF P19 CELLS AFTER SERUMWITHDRAWAL
phenylglyoxal-EPO
EPO
HELA LIVE CELL SURVIVAL
0
0.6
1
1.5
2
2.5
3
3.5
4
0
1
10
100
1000
ng/ml of compound added

Figure 14

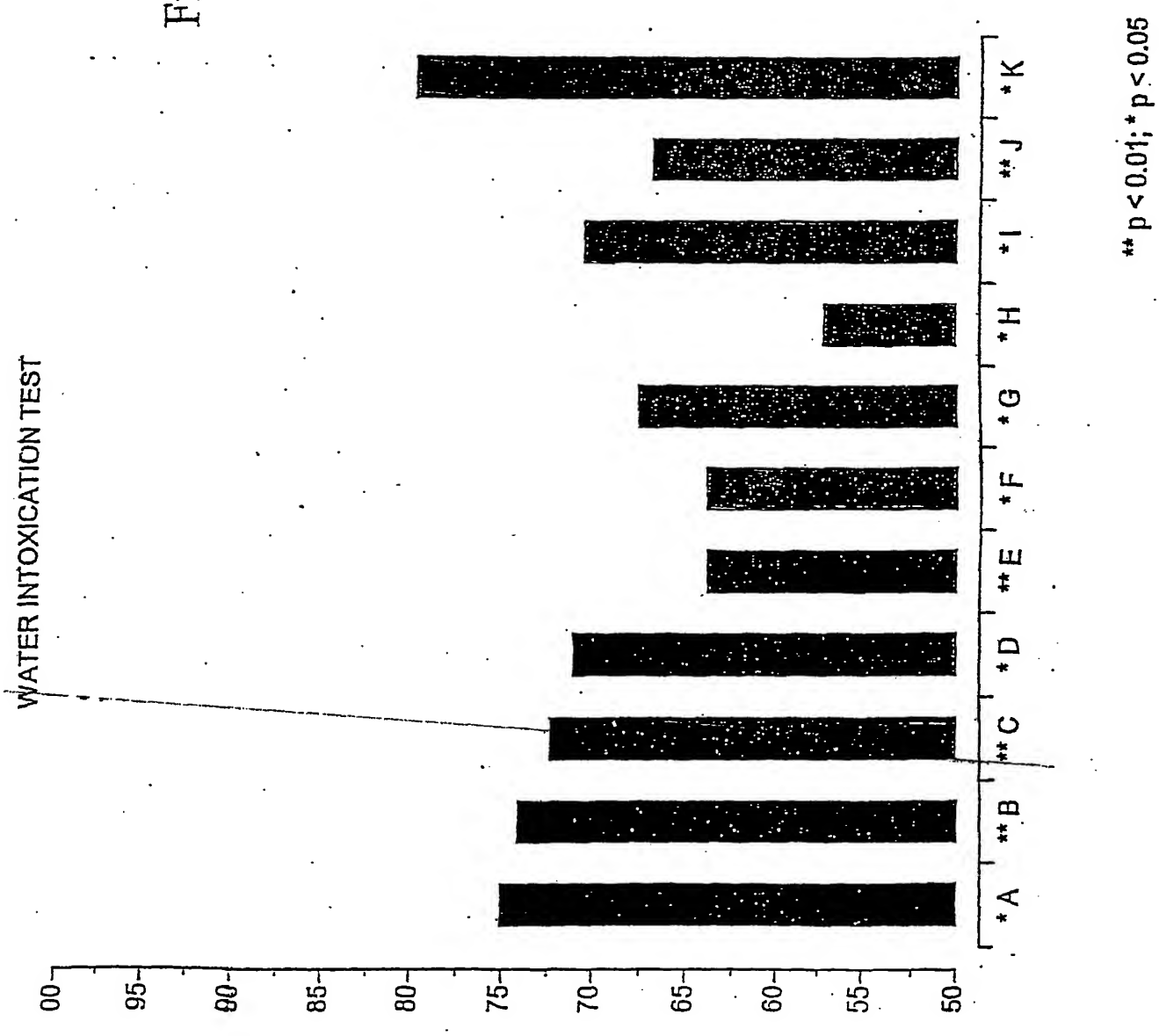

WATER INTOXICATION TEST
100
95
90
85
80
75
70
65
60
55
50
*A
**B
**C
*D
**E
*F
*G
*H
*I
**J
*K
** p < 0.01; * p < 0.05

Figure 15

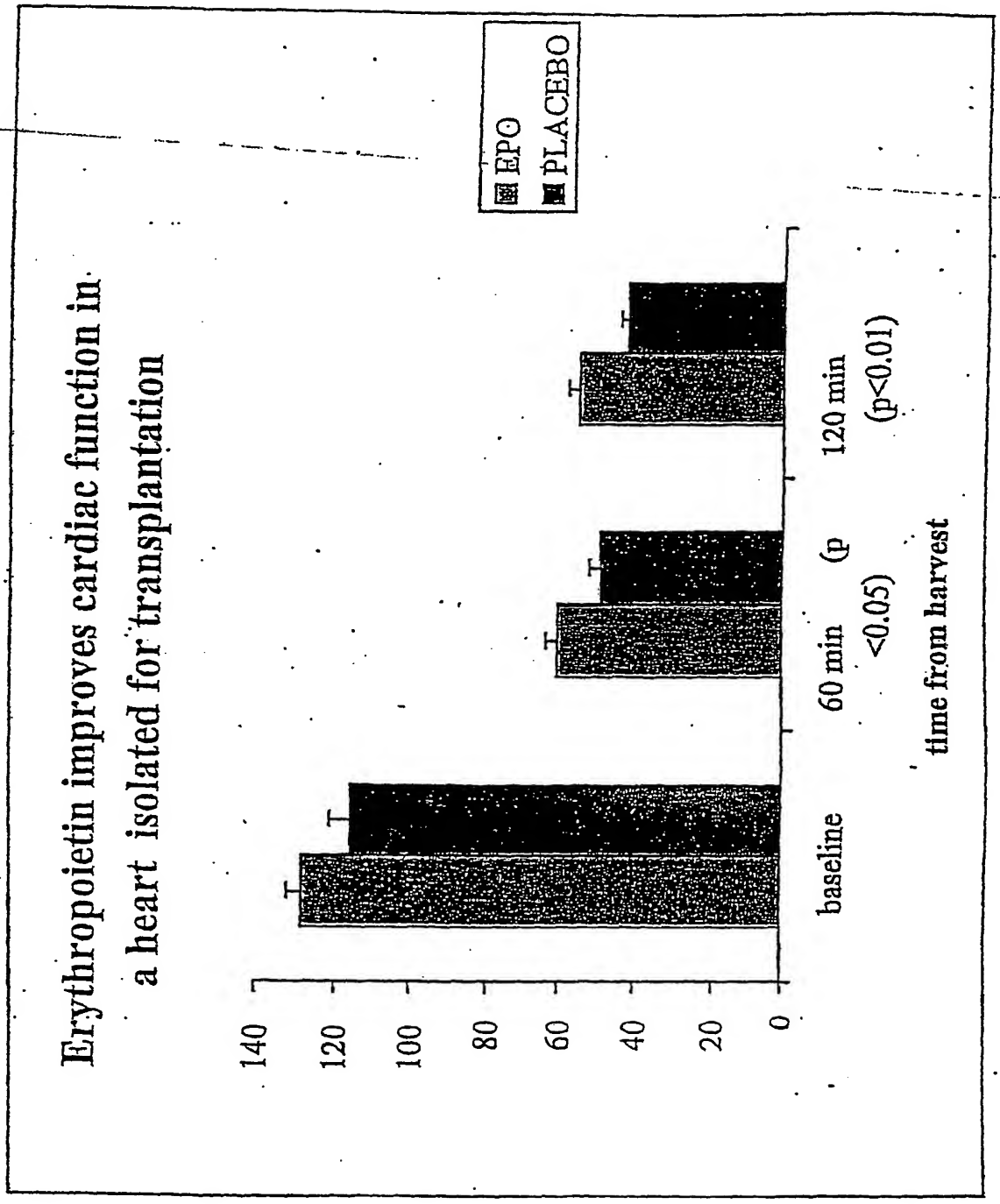

Erythropoietin improves cardiac function in a heart isolated for transplantation
EPO
PLACEBO
140
120
100
80
60
40
20
0
baseline
60 min (p <0.05)
120 min (p<0.01)
time from harvest

Figure 16

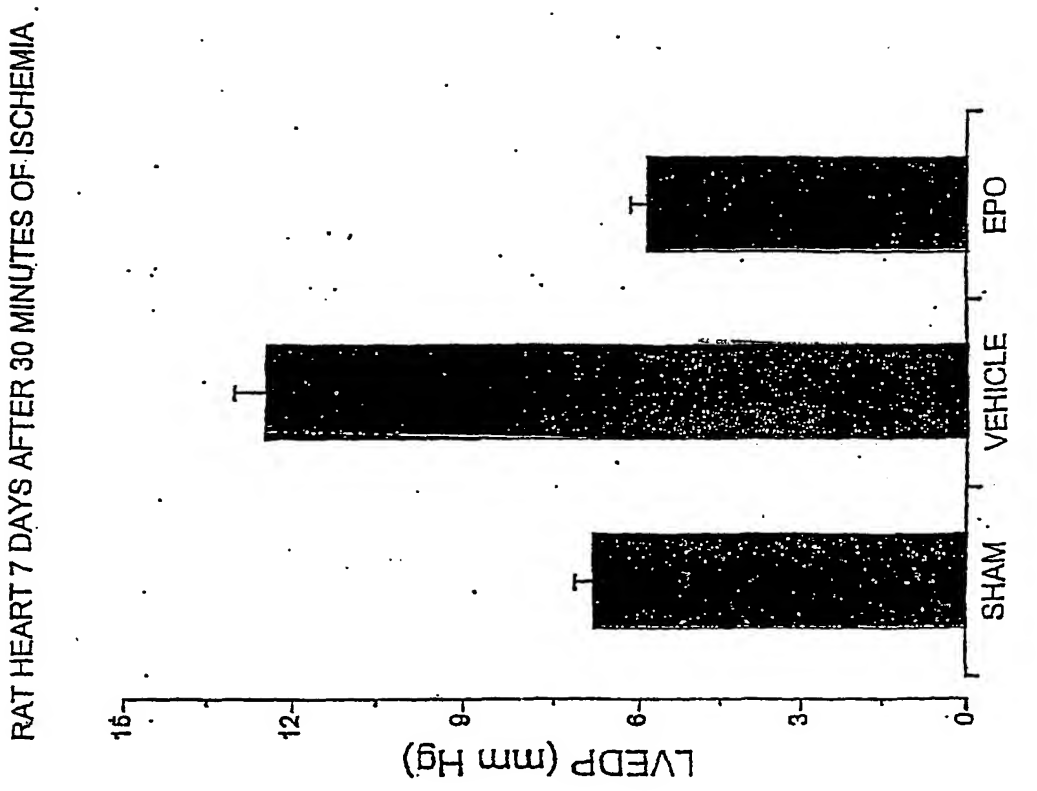

RAT HEART 7 DAYS AFTER 30 MINUTES OF ISCHEMIA
LVEDP (mm Hg)
0
3
6
9
12
15
SHAM
VEHICLE
EPO

Figure 17

Electroretinograms from rats subjected to 60 minutes of ischemia

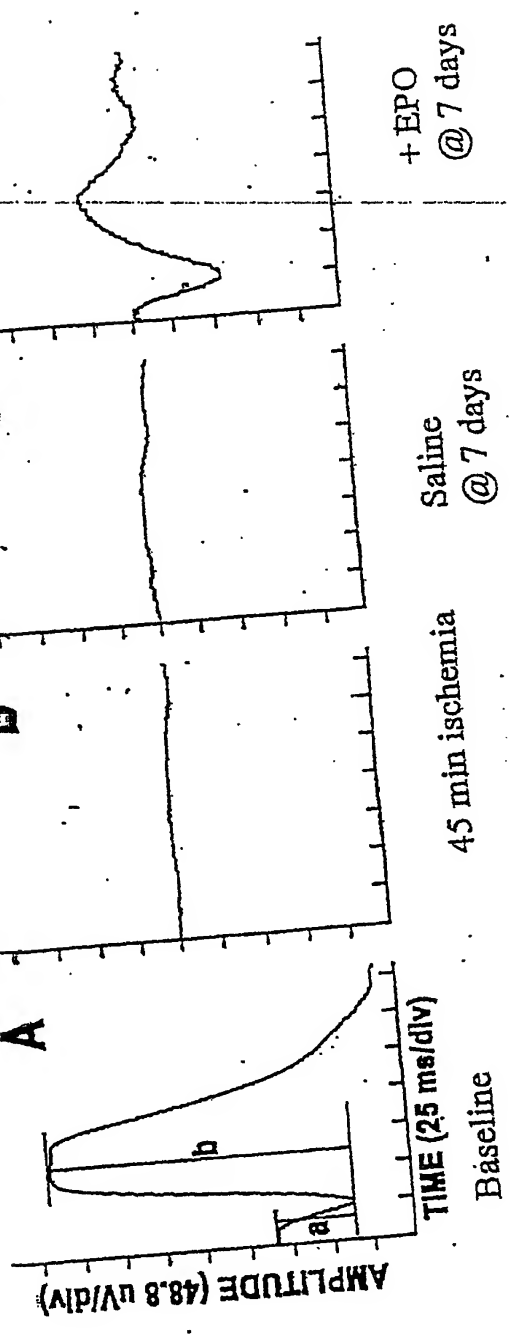


Figure 18

Retinogram amplitude after 60 min. ischemia

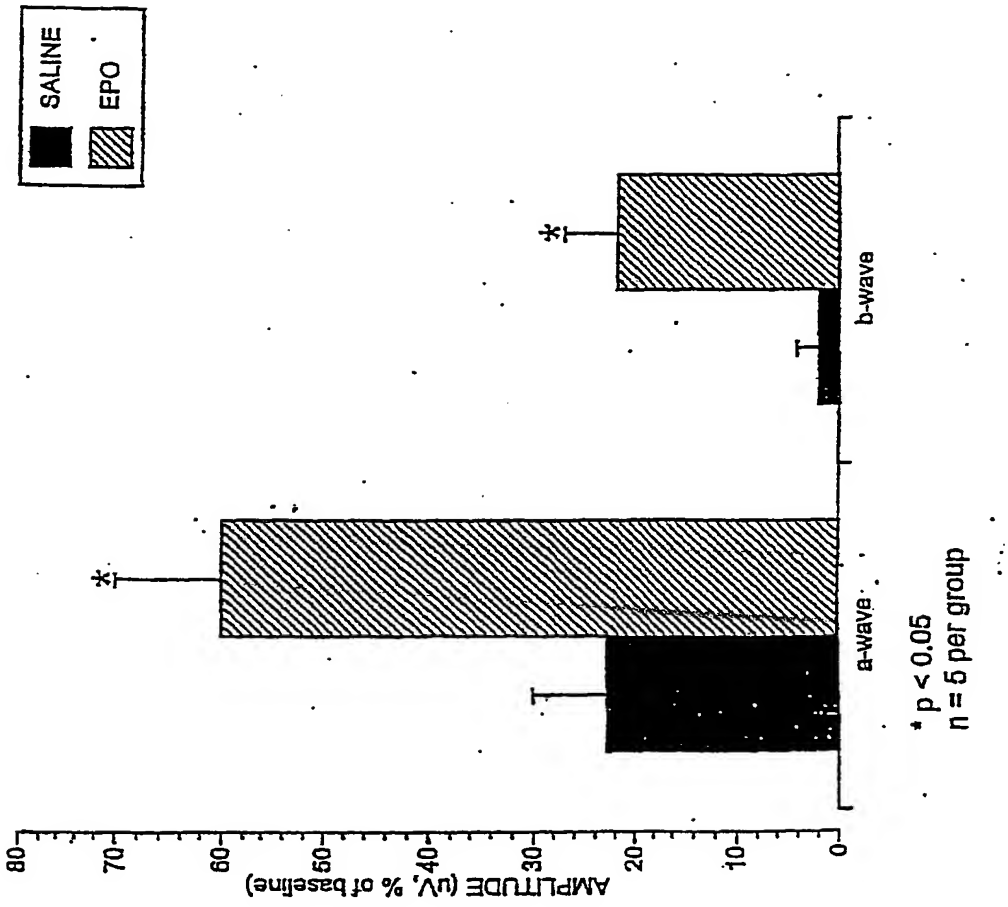


Figure 19

Morris water maze; female Balb/c mice n= 16. Blunt brain trauma with EPO rx beginning on day 5 after injury. First water maze test began 1 week after EPO dosing began (12 days after injury). Both groups of animals did poorly with swim times ~80 out of 90 seconds possible. Negative values indicates that EPO is better. Means of 4 trials per day.

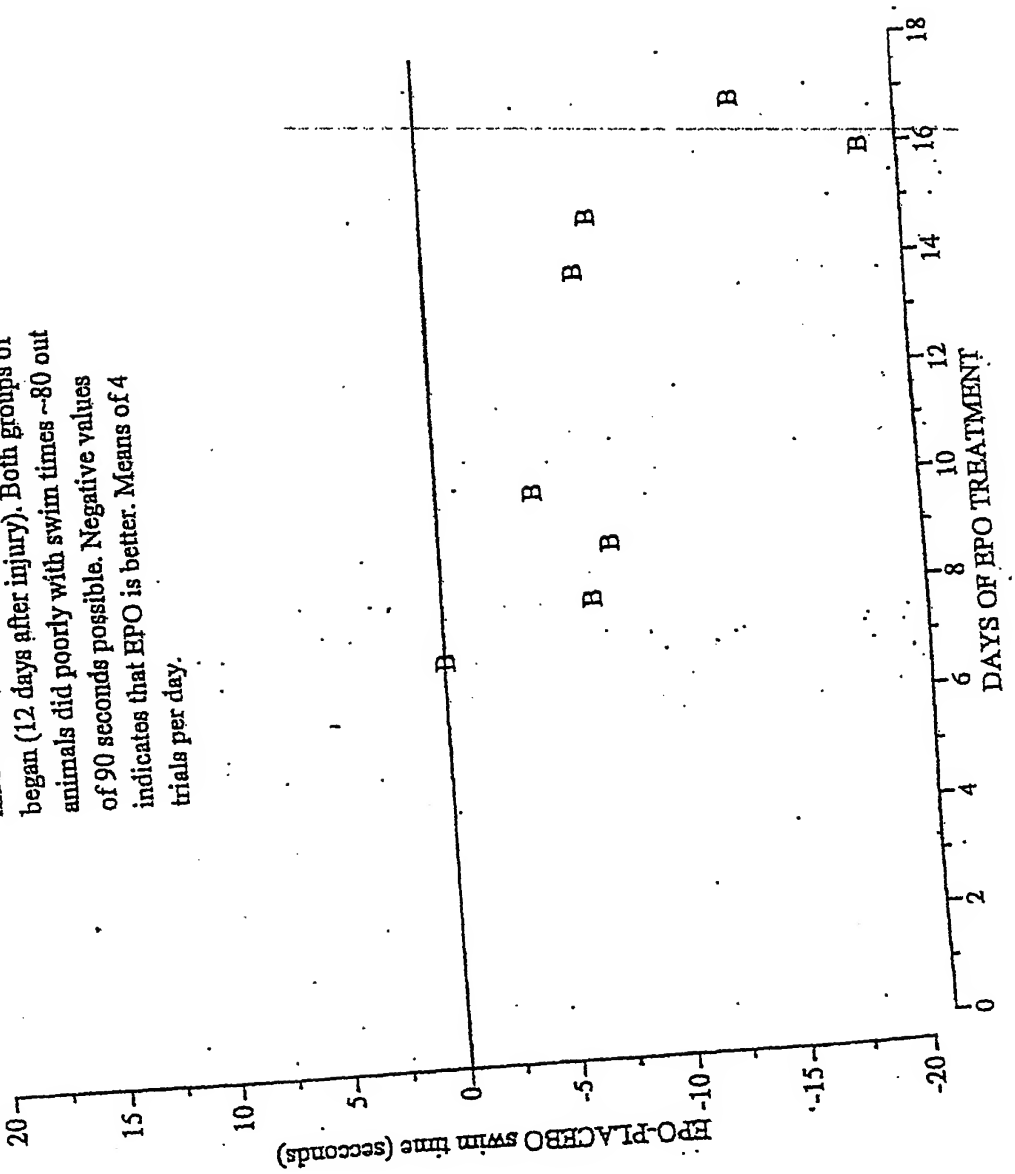


Figure 20

Difference in swimming time to platform in Morris water maze.
Bab/c mice; brain trauma 1 month previously, treated with 5000U/kg EPO daily except weekends beginning one month after injury
(n= 7 each group)
means of 4 trials each day

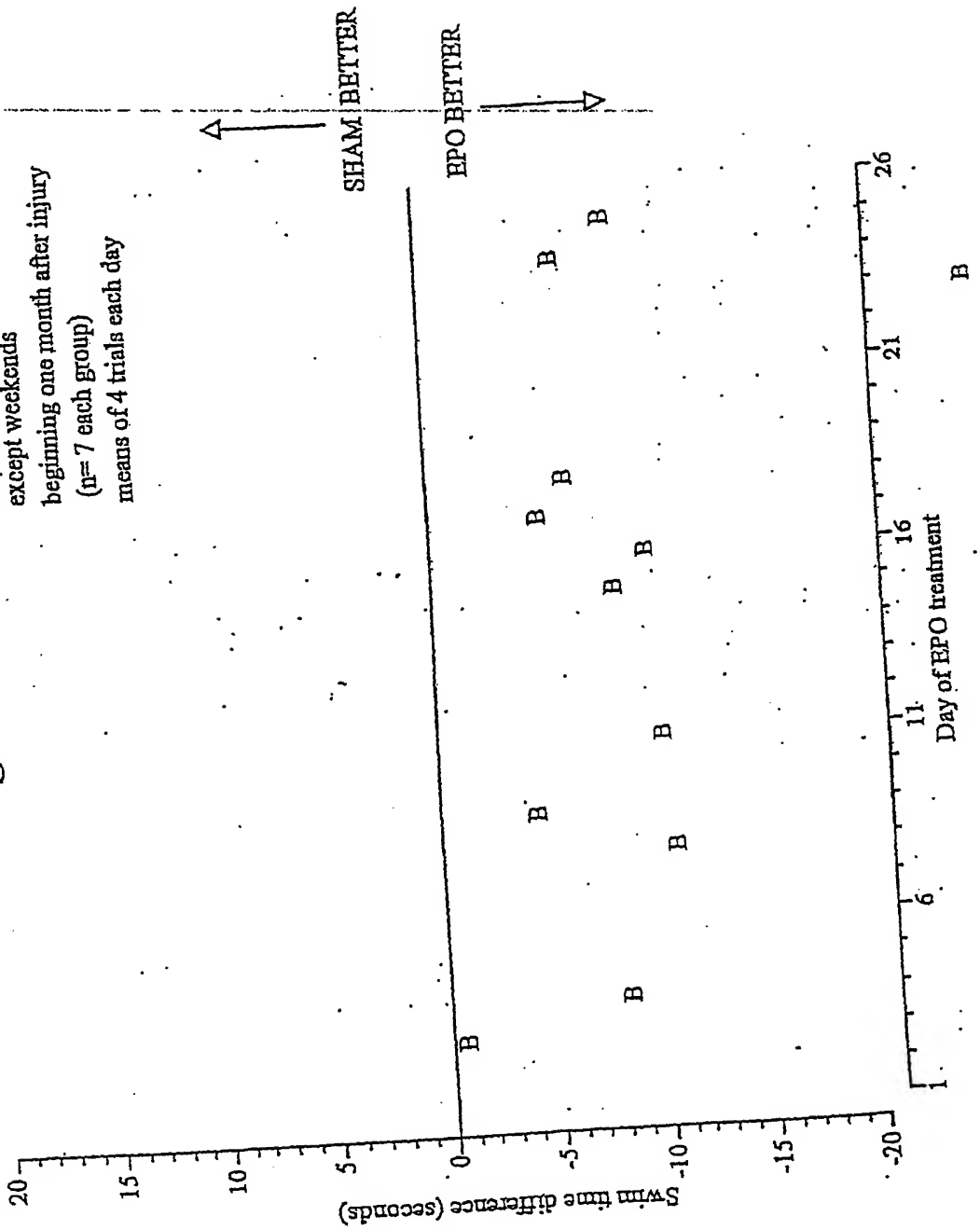


Figure 21

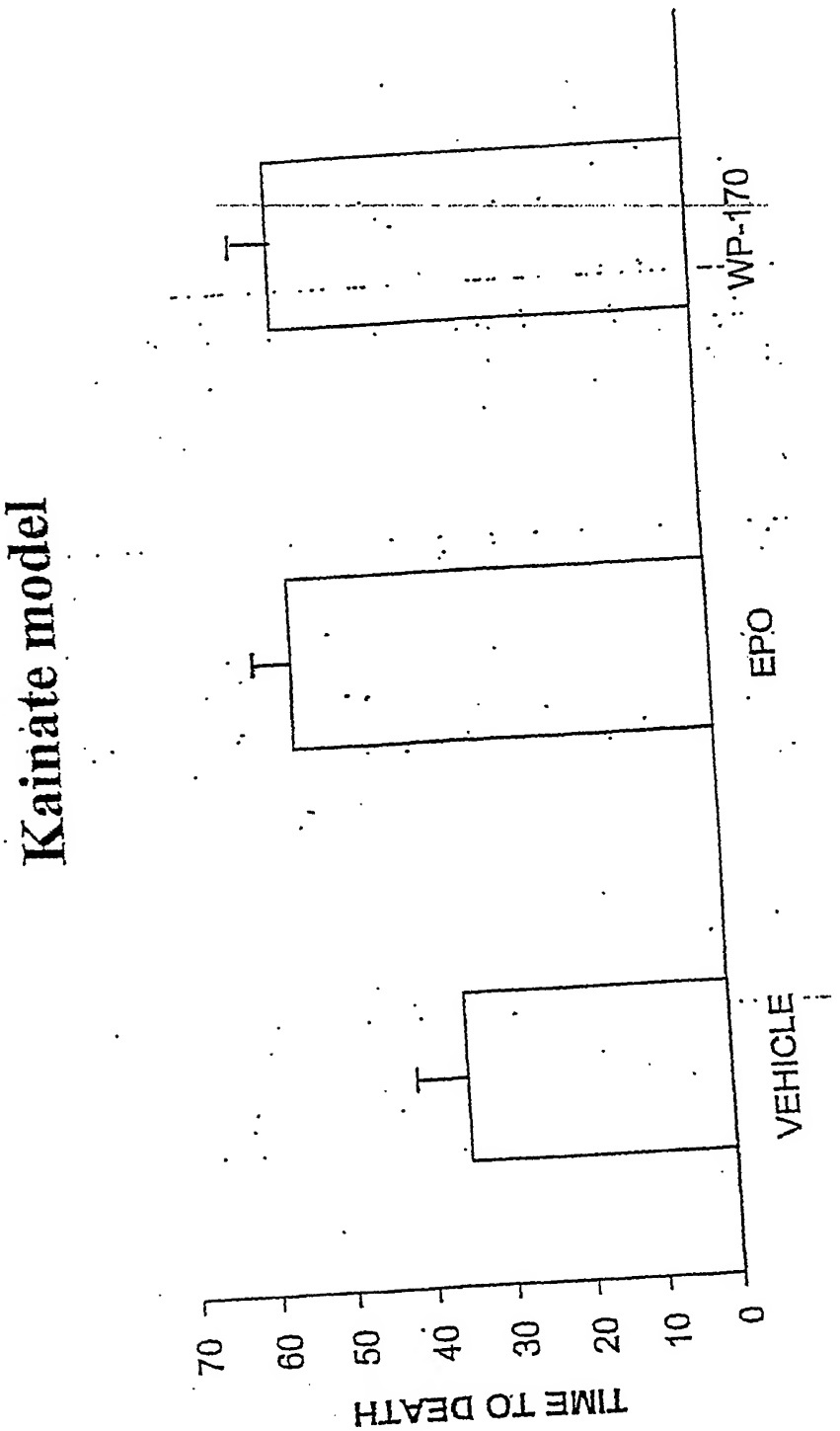

Kainate model
TIME TO DEATH
0
10
20
30
40
50
60
70
VEHICLE
EPO
WP-170

Figure 22

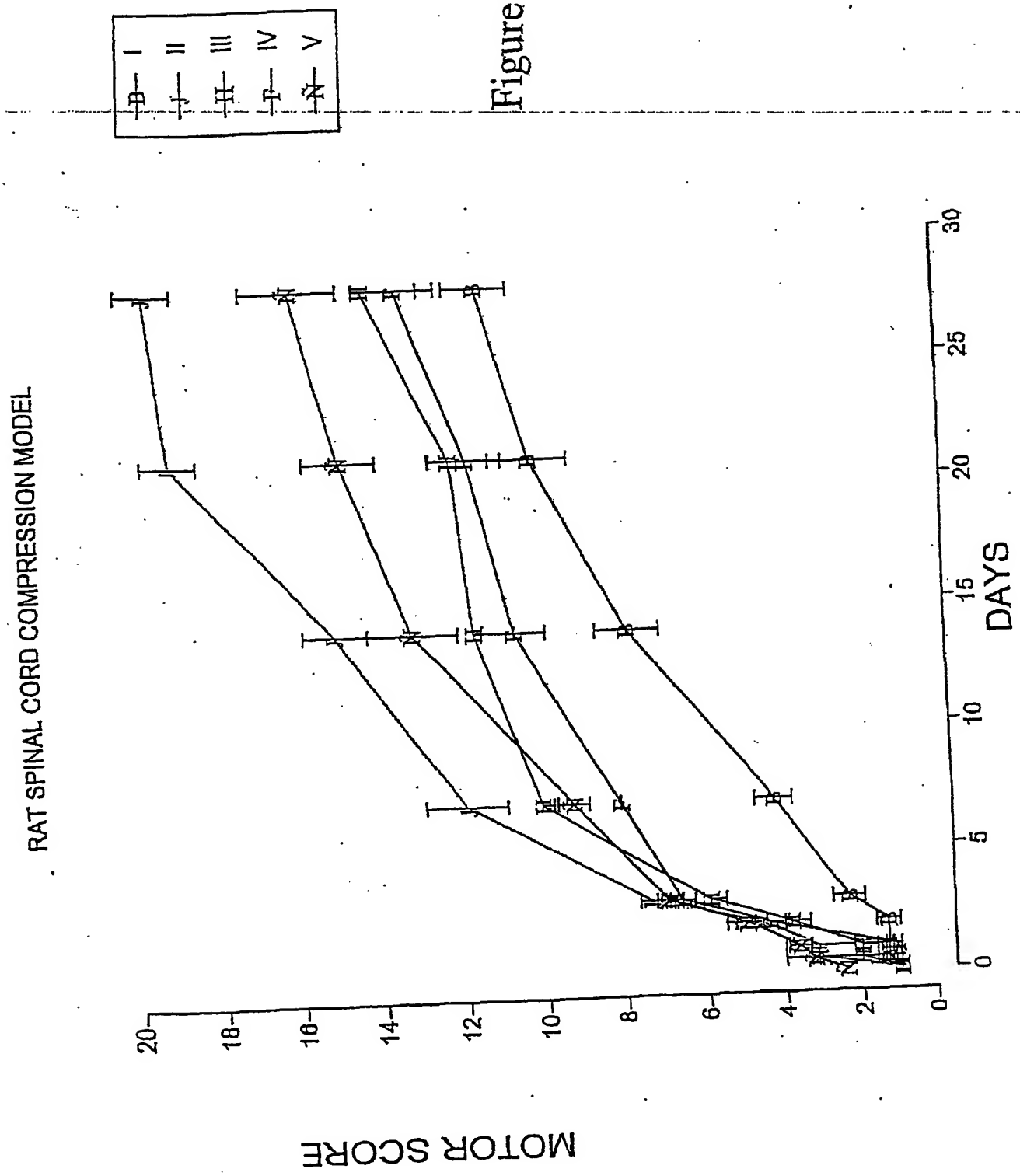
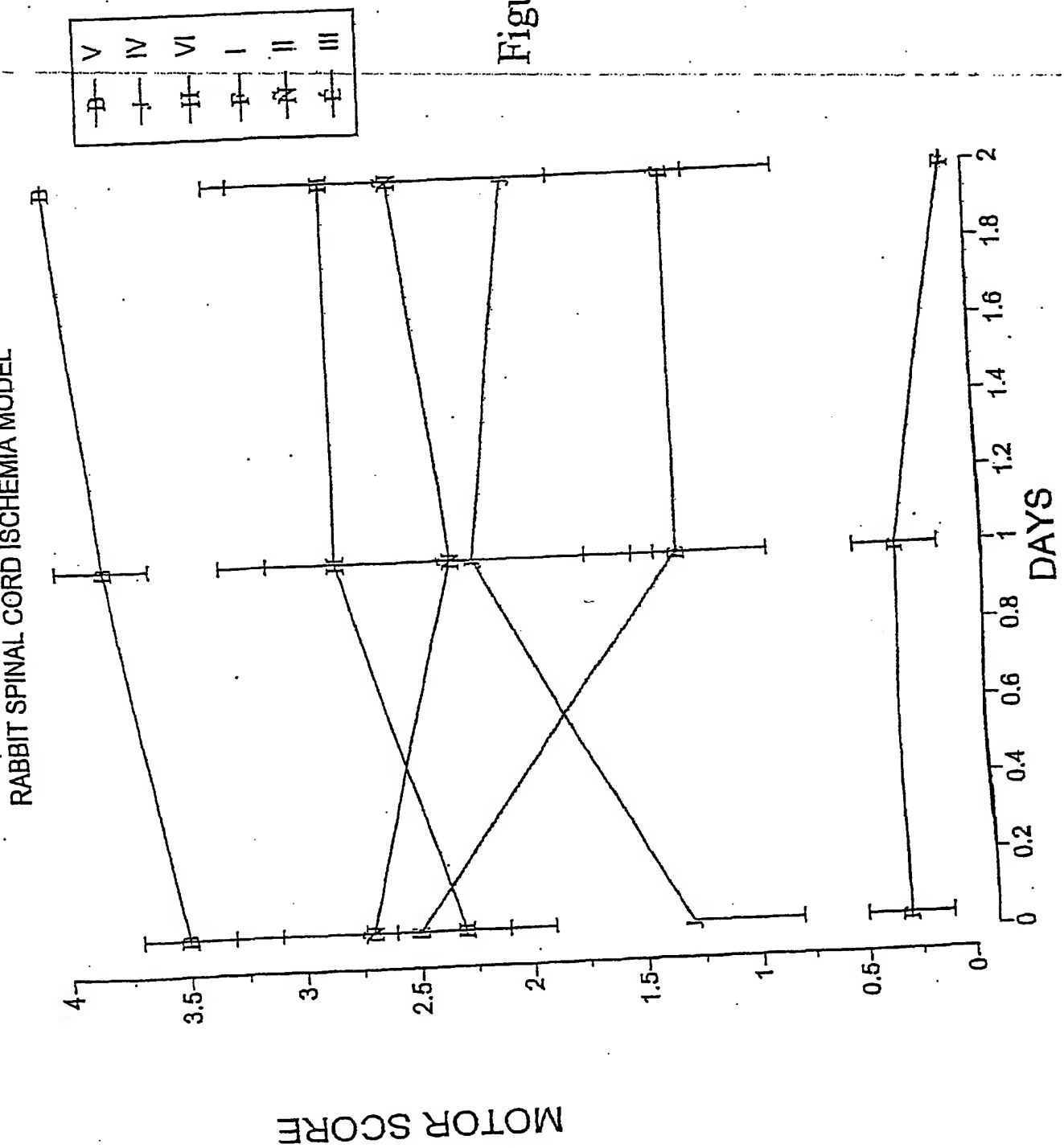

I
II
III
IV
V
RAT SPINAL CORD COMPRESSION MODEL
MOTOR SCORE
DAYS
0
2
4
6
8
10
12
14
16
18
20
0
5
10
15
20
25
30

Figure 23


RABBIT SPINAL CORD ISCHEMIA MODEL
V
IV
VI
I
II
III
MOTOR SCORE
4
3.5
3
2.5
2
1.5
1
0.5
0
DAYS
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2

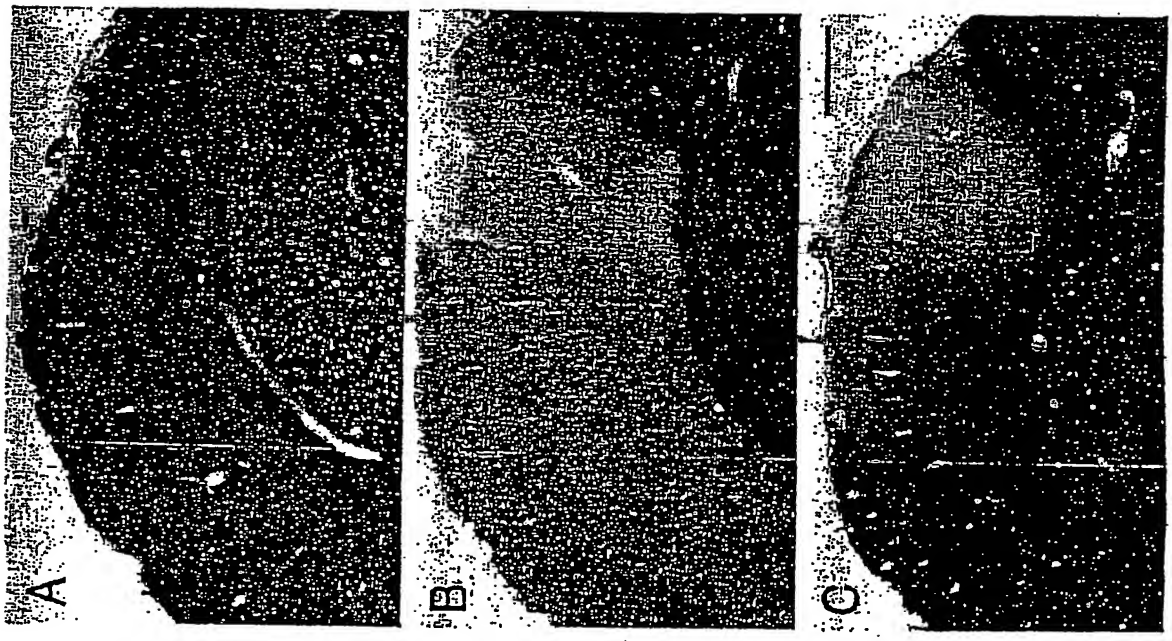


Figure 24

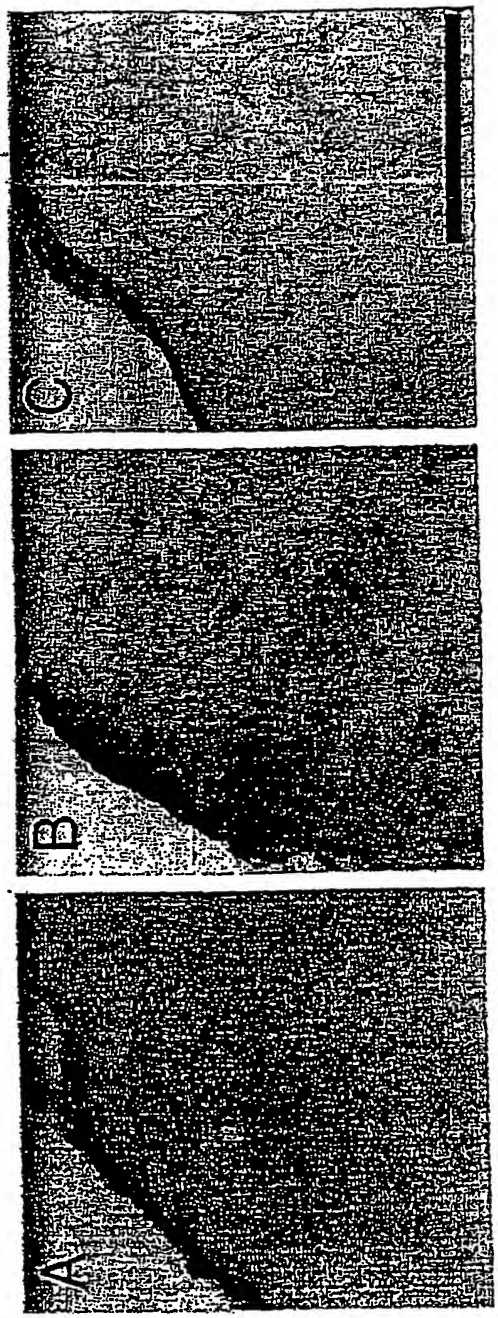


Figure 25

Figure 26

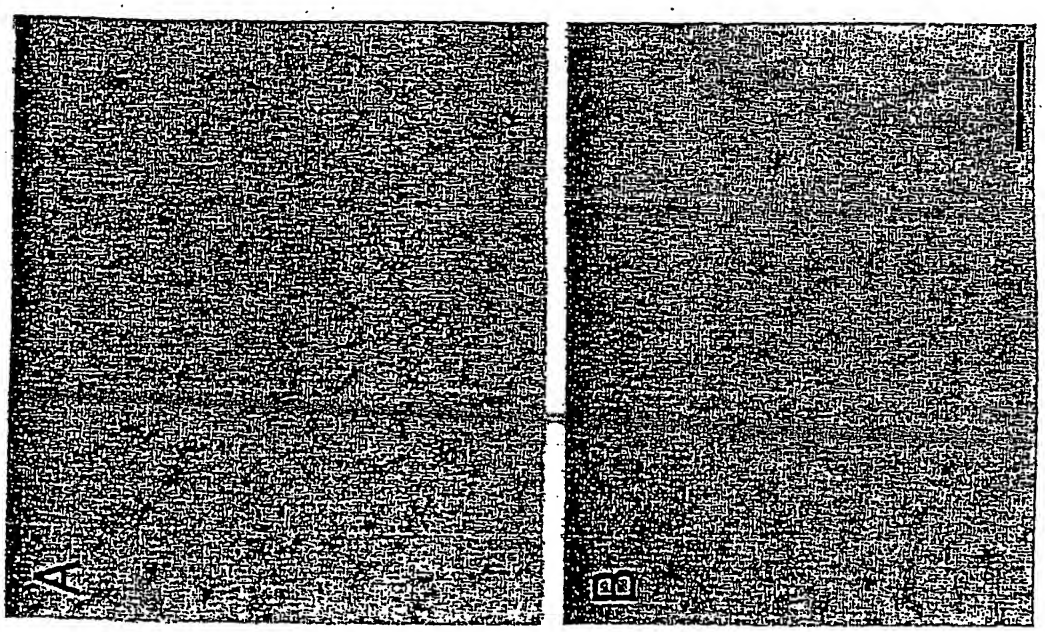
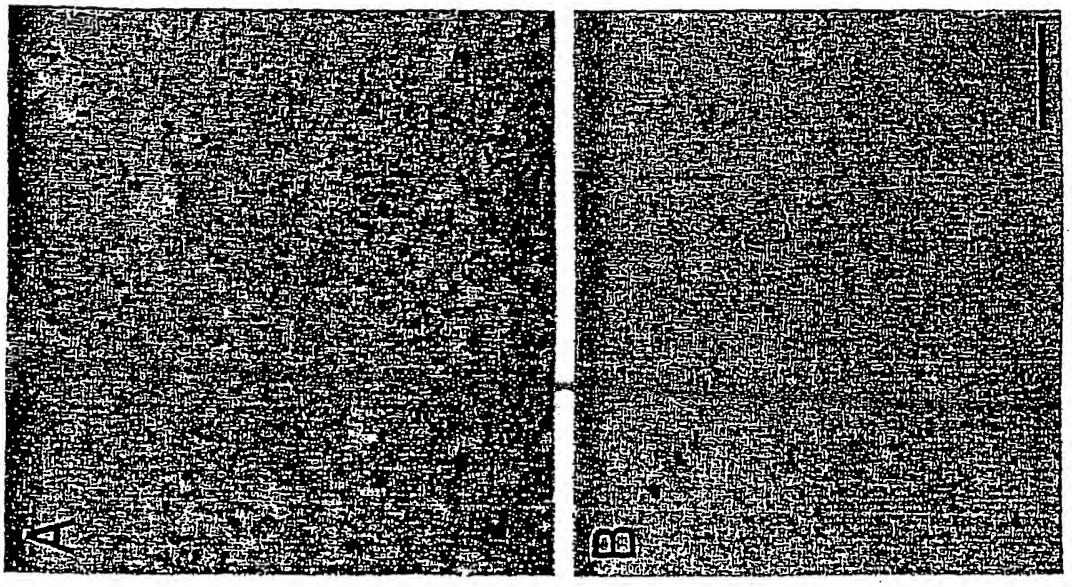


Figure 27



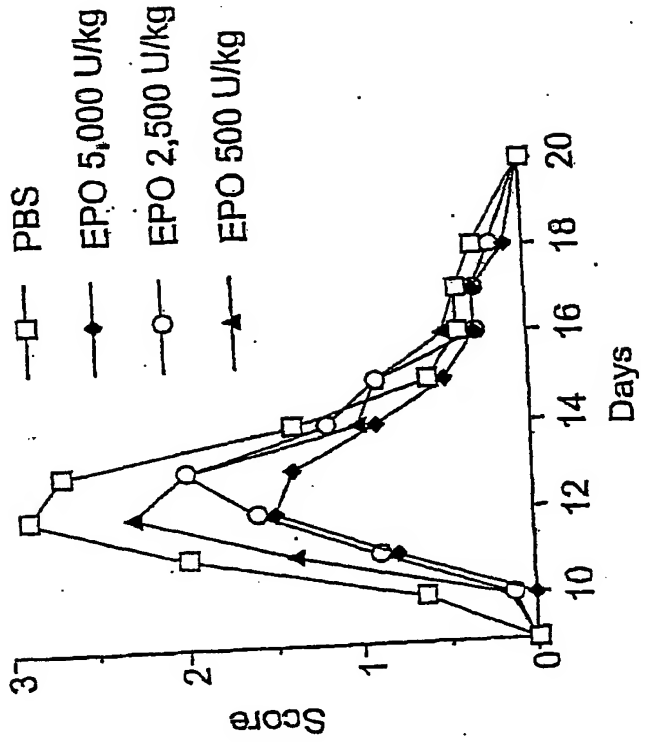
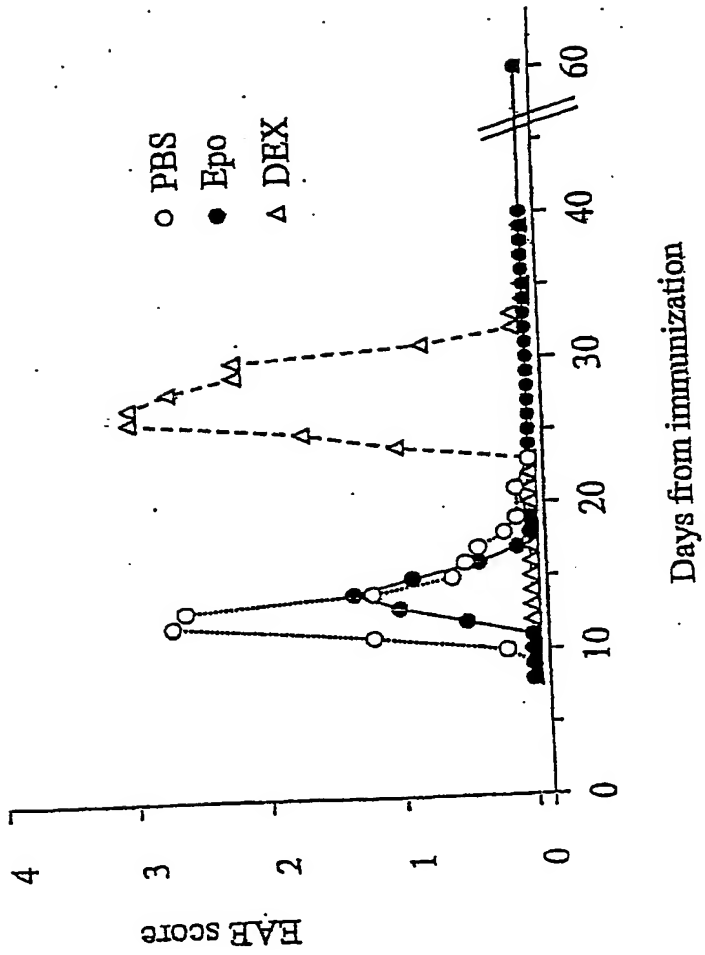

PBS
EPO 5,000 U/kg
EPO 2,500 U/kg
EPO 500 U/kg
Score
Days
0
1
2
3
10
12
14
16
18
20

Figure 28

Figure 29


PBS
Epo
DEX
EAE score
Days from immunization
0
1
2
3
4
0
10
20
30
40
60

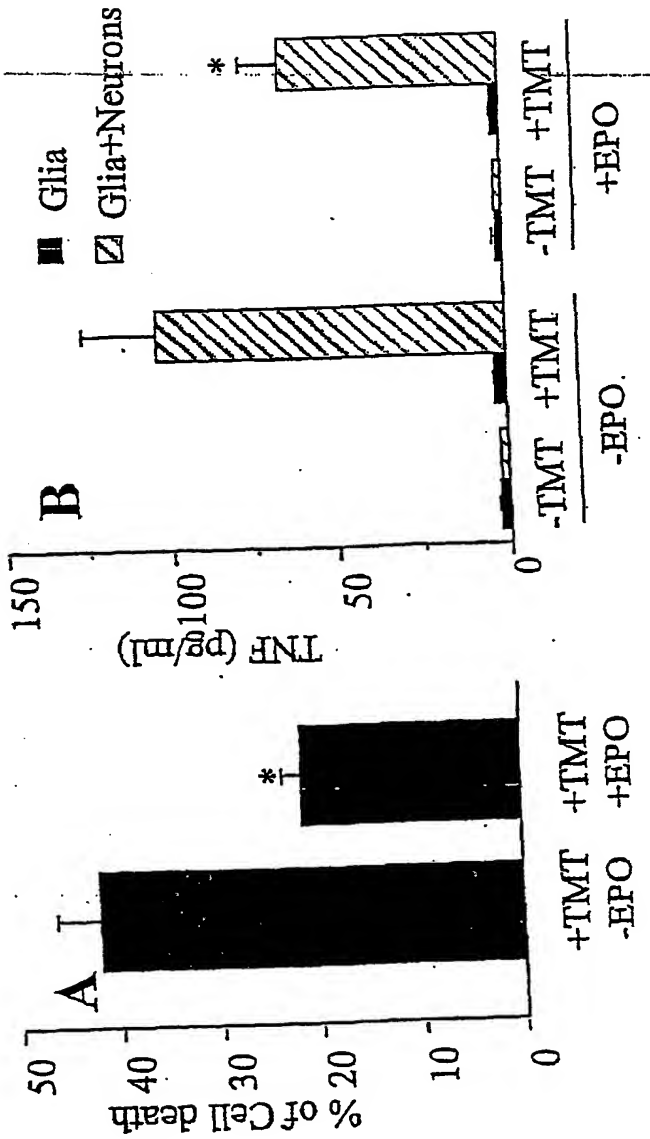

A
% of Cell death
50
40
30
20
10
0
*
+TMT +TMT
-EPO +EPO
B
TNF (pg/ml)
150
100
50
0
Glia
Glia+Neurons
*
-TMT +TMT
-EPO
-TMT +TMT
+EPO

Figure 30

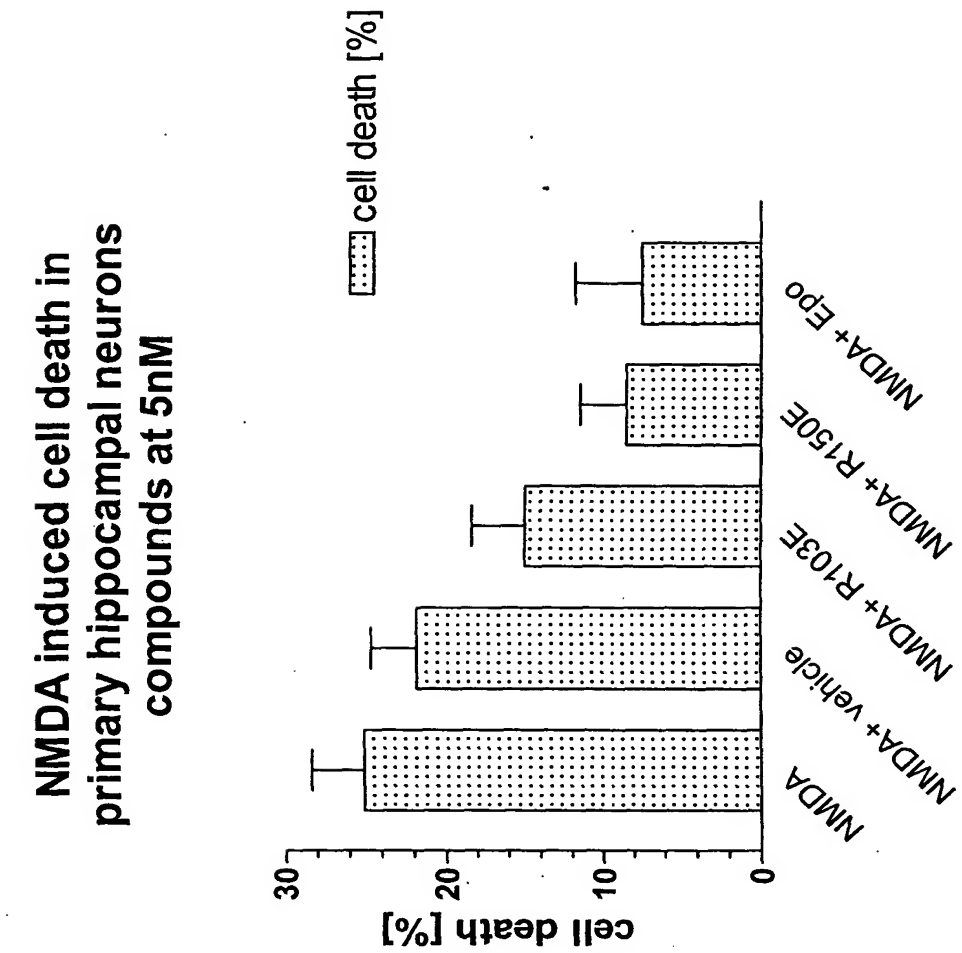

NMDA induced cell death in primary hippocampal neurons compounds at 5nM
cell death [%]
cell death [%]
0
10
20
30
NMDA
NMDA+ vehicle
NMDA+ R103E
NMDA+ R150E
NMDA+ Epo

Figure 31

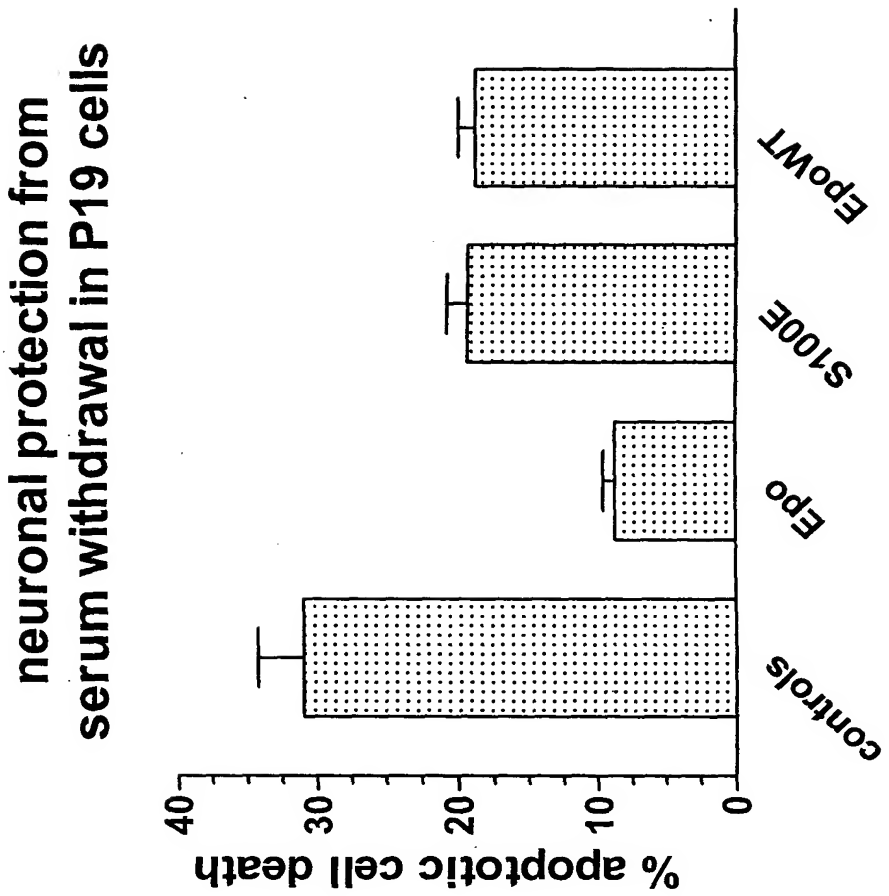
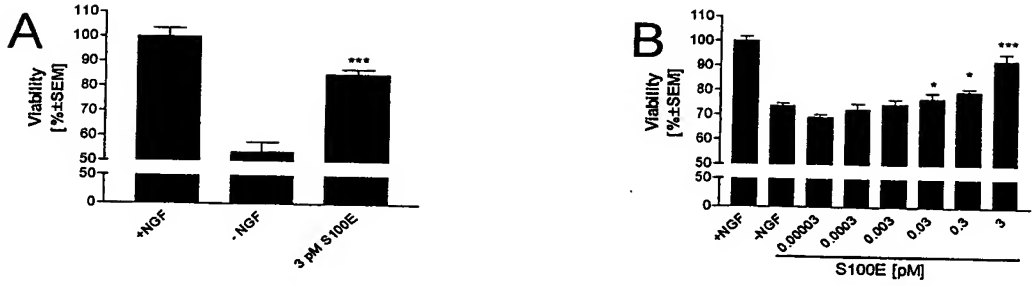
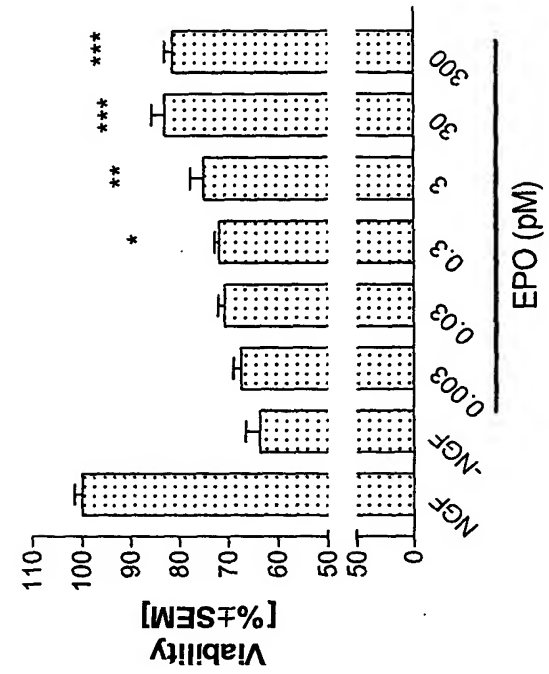
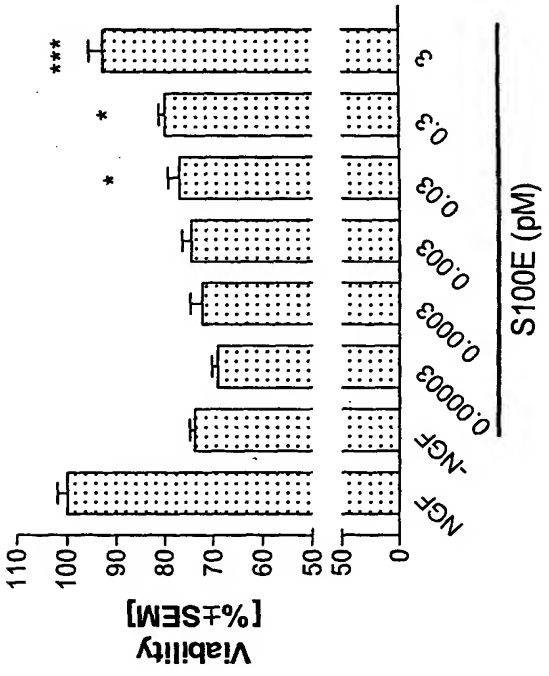

neuronal protection from
serum withdrawal in P19 cells
% apoptotic cell death
0
10
20
30
40
controls
Epo
S100E
EpoWT

Figure 32

Figure 33


A
Viability [%±SEM]
110
100
90
80
70
60
50
50
0
***
+NGF
-NGF
3 pM S100E
B
Viability [%±SEM]
110
100
90
80
70
60
50
50
0
*
*
***
+NGF
-NGF
0.00003
0.0003
0.003
0.03
0.3
3
S100E [pM]


Viability
[%±SEM]
110
100
90
80
70
60
50
50
0
NGF
-NGF
0.003
0.03
0.3
3
30
300
EPO (pM)
*
**
***
***


Viability
[%±SEM]
110
100
90
80
70
60
50
50
0
NGF
-NGF
0.00003
0.0003
0.003
0.03
0.3
3
S100E (pM)
*
*
***

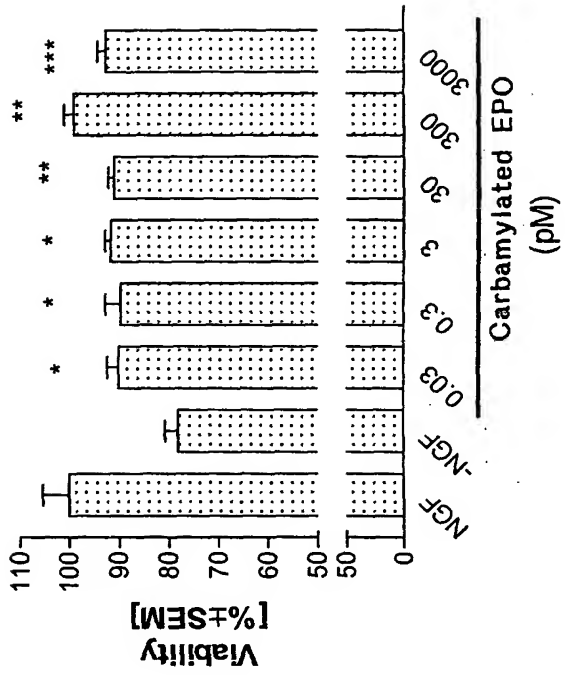
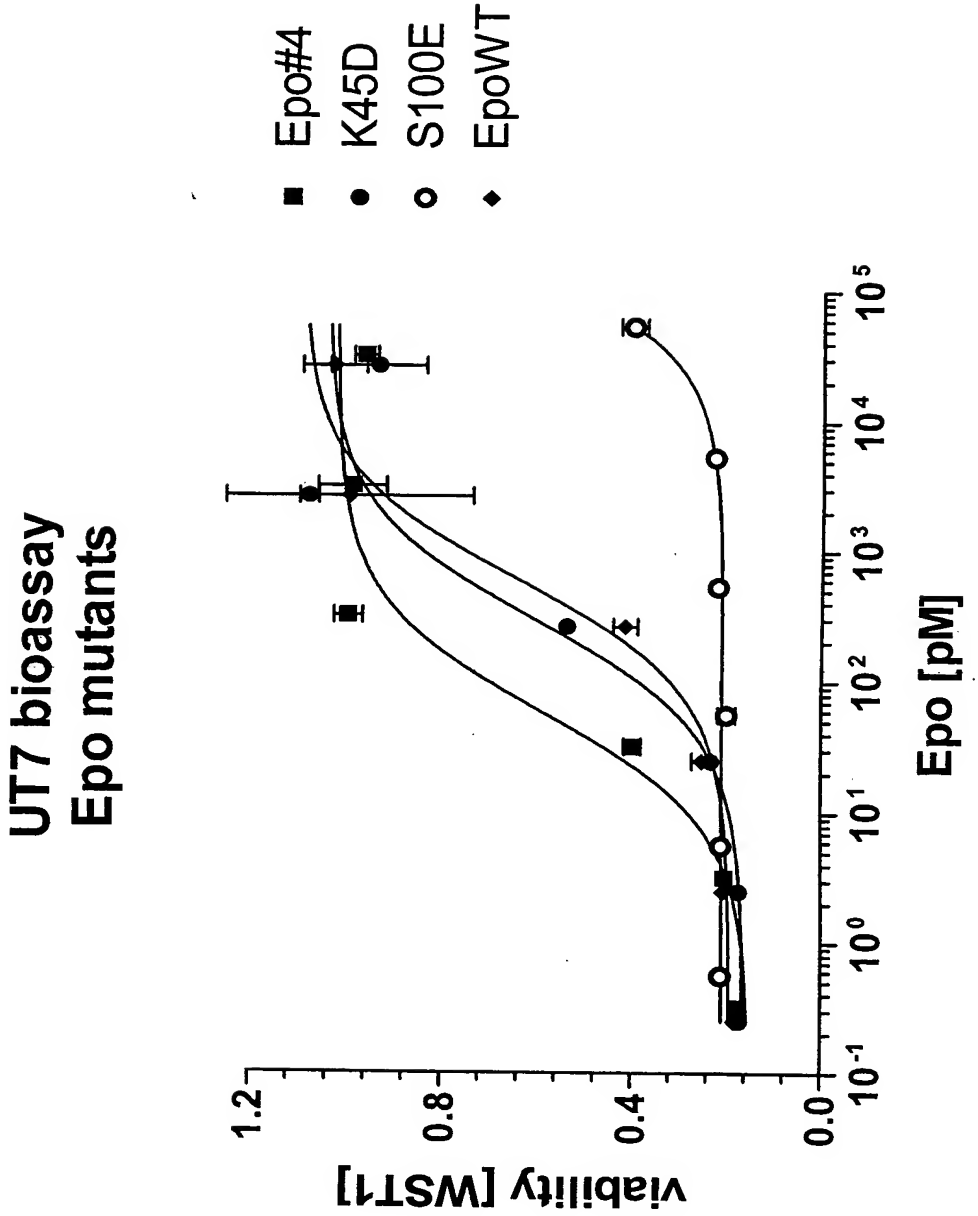
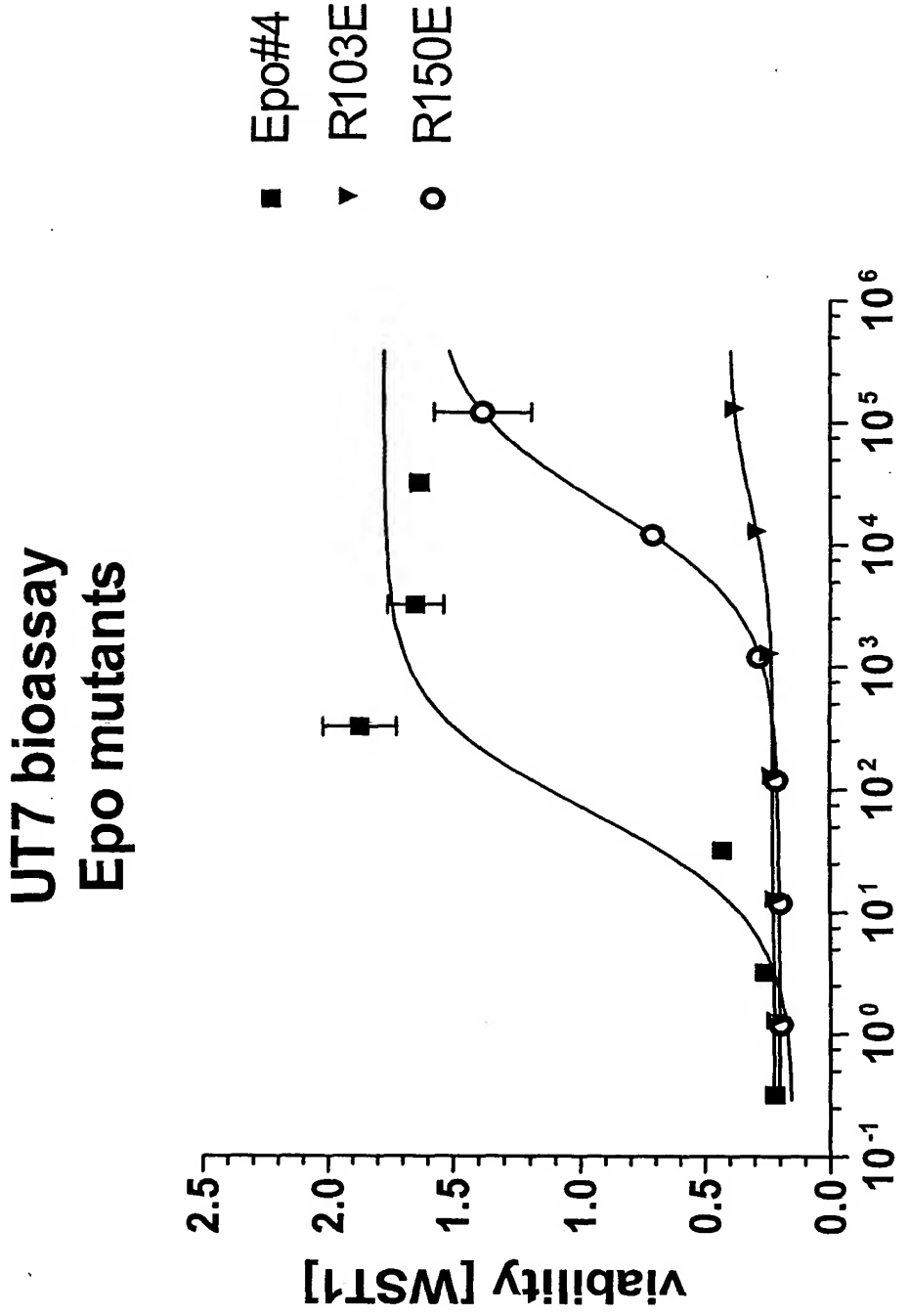

Viability
[%±SEM]
110
100
90
80
70
60
50
50
0
NGF
-NGF
0.03
0.3
3
30
300
3000
Carbamylated EPO
(pM)
*
*
*
**
**
***

Figure 34



	Epo#4	K45D	S100E	EpoWT
EC50	58.13	294.0	5.3840e+006	608.0

Figure 35



EC50 [pM]	Epo#4	R103E	R150E
	66,5	21760	20450

Figure 36

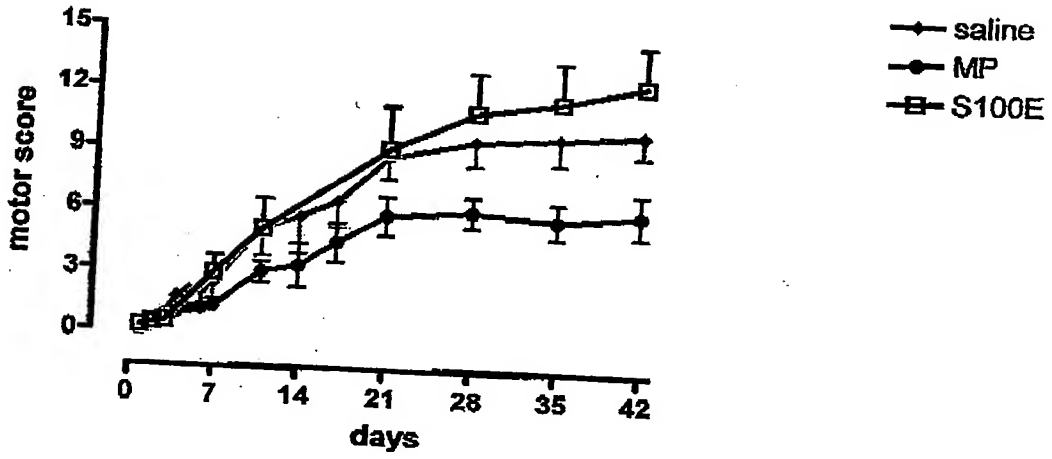
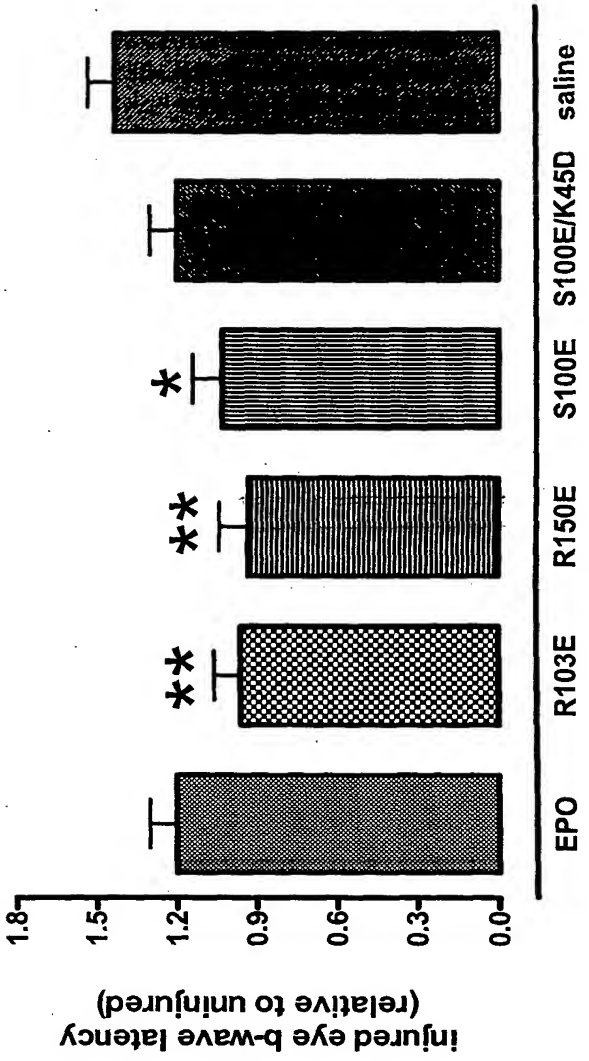


Figure 37



** p<0.01; * p< 0.05 versus saline

Figure 38